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CONTENTS.

Part 1 (March, 1915).

Part 2 (September, 1915).

Part 3 (February, 1916).

Part 4 (April, 1916).

VOLUME VII MARCH, 1915 PART I

THE ATMOSPHERE OF THE SOIL: ITS COMPOSITION AND THE CAUSES OF VARIATION.

BY EDWARD JOHN RUSSELL AND ALFRED APPLEYARD.

(Rothamsted Experimental Station.)

(With 17 Text-figures.]

Introduction.

THE remarkable relationships existing between the microorganisms of the soil and the growth of plants have given rise to numerous researches on the bacteria, fungi, and more recently the protozoa of the soil, and considerable knowledge has now been obtained of the organisms present in normal soils. The earlier investigations were necessarily confined largely to methods of isolation, descriptions of the organisms found and studies of their behaviour in certain culture solutions, but sufficient of this preliminary work has been done to enable us to attack the real problem and try to obtain a picture of the life in the soil as it actually is. For this purpose it is necessary to know the relative abundance of the various organisms, to find out which are active and which inert, and to discover what the active forms are doing and what is their mode of life. Before the bacteriological and zoological work can be fully interpreted, however, it is necessary to discover the conditions under which life in the soil goes on, and in the series of papers, of which this is the first, it is proposed to deal with the air supply, water supply, and temperature of our own soils and by comparison with other investigations to see how far the observed relationships hold generally.

In the present paper we shall confine ourselves to the atmosphere of the soil. The soil mass is porous and the volume of solid matter in our case[1] is approximately two-thirds of the whole, leaving one-third pore space. The pore space, however, is not empty but contains a considerable amount of water, and the actual space empty except for

[1] For analysis of the soil, see p. 44.

air is commonly not more than 10 to 20 per cent. of the volume of the soil. The pores appear to be continuous and seem to maintain fairly complete communication between the various layers of the soil; in some places the communication is made more effective by the presence of cracks and burrows.

The soil atmosphere is the air present in these pores. Its biological significance lies in the fact that it is the air surrounding the soil organisms and the roots of plants, and is either in actual contact with them or is separated from them only by a thin film of water or colloidal matter. It is obviously part of the ordinary atmosphere but its composition is influenced by two causes: oxygen is absorbed and carbon dioxide produced by the inhabitants of the soil; while on the other hand, diffusion and other processes of gaseous interchange are constantly replacing it with ordinary atmospheric air, thus eliminating any differences in composition brought about by biochemical or other changes. As a net result the composition of the soil air at any moment is determined by the difference of velocity with which these two processes take place.

Unfortunately the mechanism of gaseous interchange in the soil is not sufficiently well known to enable us to ascertain the speed at which it goes on and so to discover the rate of production of carbon dioxide, a quantity of great importance in the study of the biochemical changes in the soil, but we have obtained evidence that our curves are mainly determined by the production and not by the loss of carbon dioxide from the soil. In any case for our present purpose of discovering the conditions under which life goes on in the soil it is mainly necessary to know the resultant of the various actions concerned.

Preliminary determinations showed that it is not difficult to draw a sample of gas from the soil, that is fairly representative of the soil air and is uncontaminated by atmospheric air. In our experiments the depth selected has been 6 inches, this being right in the region where the soil changes take place, besides being convenient for working. But as a matter of fact no great difference in composition was found on going somewhat deeper: thus the following results (Table I) were obtained at 6 and 18 inches respectively.

In general the soil air was found to be very similar in composition to ordinary atmospheric air, especially as regards the percentages of oxygen and of nitrogen. It commonly contains less oxygen and more carbon dioxide, usually also more nitrogen, but the differences are often small and only detected with certainty by careful analyses (Table VI).

TABLE I. *Comparison of composition of soil air taken from a depth of 6 and 18 in. in the soil. 30 January 1914. Percentage by volume.*

	CO_2		O_2	
	6″ deep	18″ deep	6″ deep	18″ deep
Grassland, Greatfield	1·46	1·64	18·44	17·87
Arable land, Broadbalk (dunged plot) ..	0·34	0·50	20·52	20·33
Arable land, Broadbalk (unmanured plot)	0·34	0·45	20·32	20·35

Unlike atmospheric air, however, the soil air is not constant in composition but changes somewhat from day to day and even on the same day at different spots in the field; nevertheless the values fall within fairly narrow limits.

There are two kinds of variation in composition; the local daily ones just referred to, and the greater variations produced by season, treatment, etc.: the latter may be so great as to mask altogether the local fluctuations. In our experiments the greatest factor of all was the effect of season. Whatever the history of the soil its atmosphere in spring and to a less extent in autumn was characterised by high amounts of carbon dioxide indicating rapid biochemical changes at these seasons of the year, while in summer and winter the amounts were much lower. The effect is complex and includes at least two others each of which was found to be very potent: the temperature during the period December to June, and moistness of the soil during part of the summer months. (Figs. 7 and 8.)

In addition there is the possibility that a certain amount of partial sterilisation has taken place during the winter and during the dry summer, leading to considerable bacterial activity immediately conditions become favourable once more.

This seasonal effect dominates all the others and impresses on all the curves the same general type seen in Figs. 1–6[1]. Other factors, such as manuring, cropping, etc., simply raise or lower the whole curve according as they give rise to more or less carbon dioxide; in particular the effect of the crop proved to be considerably less than was anticipated.

Within these major variations there fall the smaller fluctuations

[1] See Table VI for data.

attributable to differences in composition of the soil[1], especially the distribution of organic matter, organisms, plant roots and passages such as cracks, burrows of earthworms, etc.; to daily changes in temperature and moisture content of the soil, or to any cause that would facilitate interchange between the soil air and the atmosphere. These local and daily fluctuations lie between relatively narrow limits, and by taking a mean of a number of samples it is not difficult to arrive at a value that approximately expresses the composition of the soil air at the time. Some of these values are given in Table II.

TABLE II. *Mean composition of soil air from various Rothamsted plots. Percentage by volume.*

	CO_2	O_2	N_2
Arable land manured (farmyard manure) and cropped			
Broadbalk wheat Plot 2 (Summer	0·23	20·74	79·03
Broadbalk wheat Plot 2 (Winter	0·37	20·31	79·32
Arable land unmanured and cropped			
Broadbalk wheat Plot 3 (Summer	0·19	20·82	78·99
Broadbalk wheat Plot 3 (Winter	0·21	20·42	79·37
Arable land unmanured and cropped Hoos wheat			
Summer	0·28	20·65	79·07
Winter	0·20	20·71	79·09
Arable land unmanured and cropped Hoos fallow			
Summer	0·12	20·84	79·04
Winter	0·08	20·78	79·14
Mean of all the arable soils	0·25±0·1	20·6±0·2	79·2±0·2
Pasture land. Winter	1·57	18·02	80·04
Atmospheric air	0·03	20·97	79·00

The column labelled nitrogen is simply the residual gas after the carbon dioxide and oxygen have been removed in the analytical process and it includes other gases just as in the case of atmospheric air. Sir James Dewar kindly examined some of the samples for hydrogen, but found only quantities of the same order as in the atmosphere, while our own tests have failed to reveal appreciable quantities either of

[1] We are here using the word to denote the whole of the surface soil complex: solid matter, water, air spaces, etc. It is unfortunate that no soil chemist has yet had the courage to coin a word to express this meaning. The word "soil" is ambiguous, as it means also the actual solid matter.

methane or any other combustible gas. We may therefore safely assume that the residual gas is practically all nitrogen.

This then represents the ordinary composition of the air filling the pores of the soil at a depth of 6 inches, the layer within which most of the important soil changes go on. As already pointed out it is very similar to ordinary atmospheric air but there are three important differences which may have much greater effects than would at first be expected:

1. The amount of carbon dioxide though low in the absolute, is nevertheless about ten or more times as high as in atmospheric air.

2. The amount of moisture present in the soil air is greater than in atmospheric air and is usually nearer the saturation point.

3. The soil air is still, there being much less opportunity for actual movement than in the atmosphere.

It is outside our present subject to discuss the effects of these characteristics and we need only indicate a few ways in which they may be expected to act.

There is considerable evidence that microorganisms are very sensitive to the medium in which they are placed, and the relatively high proportion of carbon dioxide in the soil atmosphere is likely to affect their activity. It is therefore necessary to take this factor into account before applying to the soil any deductions from bacteriological investigations made in the laboratory under ordinary atmospheric conditions.

In consequence of its stillness and its intimate contact with the moist soil particles, the soil air is likely to be saturated or nearly saturated with water vapour, and this condition is known to be favourable for organisms and to reduce the need for free liquid water.

The effect of the extreme stillness of the air, however, cannot be gauged; physiologists recognise that movement in the air is necessary for the comfort and well being of humans, and we should no doubt find the soil atmosphere intolerable from this cause alone, but it is difficult to form any estimate of its effect on microorganisms.

But this free air filling the pore spaces is not the only air in the soil. During the course of other experiments we had occasion to evacuate flasks containing soil, and we found that the vacuum persistently began to fall soon after exhaustion appeared to be complete. Gas was being evolved from the soil, but it came out only very slowly even when a good mercury pump was kept at work for several days.

The total amount of gas given up is not great; its characteristic feature is the absence of oxygen (except in small quantities) and the high proportion of carbon dioxide.

Some of the samples obtained had the composition shown in Table III.

TABLE III. *Composition of gas held absorbed by soil. Percentage by volume.*

	Weight of soil used, grms	Percentage of Moisture	Approximate volume of gas removed in successive extractions	Percentage composition of gas		
				CO_2	O_2	N_2
Pasture soil	352	28	1st 30 c.c.	52·0	0·7	47·3
			2nd 30	84·8	0·2	15·0
			3rd 22	99·1	0·2	0·7
Soil covered with vegetation (Broadbalk wilderness)	400	22	1st 30 c.c.	19·3	5·5	75·2
			2nd 30	57·0	2·6	40·4
			3rd 15	98·7	0·2	1·1
Rich garden soil	468	20	1st 30 c.c.	89·5	0·2	10·3
			2nd 30	99·3	0·0	0·7
			3rd 15	94·4	0·0	0·6
	extracted later		4th 30	96·8	0·0	3·1
			5th 30	92·3	0·0	7·6
Arable soil Broadbalk .. dunged plot	—	24	1st 30 c.c.	10·8	4·4	84·8
			2nd 30	57·9	1·8	40·3
			3rd 15	98·4	0·0	1·6
Broadbalk unmanured ..	497	16	1st 30 c.c.	6·3	15·1	78·6
			2nd 25	40·2	9·7	50·1

It will be observed that the composition varies with the pressure, and that the first samples withdrawn contain more oxygen than the last: the final samples are almost pure carbon dioxide.

The volume of gas obtainable depends on the amount of moisture in the soil as it is brought in from the field, and decreased as the soil becomes dryer; from which we may infer that the gas is partly dissolved in the soil moisture, though part may be dissolved in other soil constituents.

Thus it appears that there are two atmospheres in the soil: one present as free gas filling the pores, and practically as rich in oxygen as ordinary air, the other dissolved in the surface films of water and other substances, almost devoid of oxygen and consisting mainly of carbon dioxide with some nitrogen.

It is hardly likely on physical grounds that these atmospheres are abruptly parted at the surface of the film; it is more probable that the free air changes in composition at the surface of the particles where a thin layer of it is to some degree in equilibrium with the dissolved air. The stillness of the soil air is favourable to the formation of a stratum different in composition from the bulk and merging insensibly into it.

The very small amount of oxygen in the dissolved gas is evidence that the rate of consumption of oxygen in the solution is greater than the rate at which fresh supplies come in from the soil air, a fact of great biochemical significance. But still more important for our present purpose is the fact of the existence of this atmosphere almost devoid of oxygen.

We are accustomed to think of a drained cultivated soil as being under essentially aerobic conditions, and the analyses of the free air show that this view is correct. But the existence of this second atmosphere enables an organism that wants anaerobic conditions to find them by submerging itself into the medium in which this atmosphere is dissolved, especially if at the same time it associates itself with an aerobic form capable of taking up any oxygen that becomes dissolved. Thus alongside of the aerobic life in the soil there is the possibility of anaerobic life, and we can no longer dismiss a possible soil change as unlikely simply on the grounds that it requires anaerobic conditions. In the present paper we confine ourselves to the free air in the soil but hope to deal with the dissolved air later on.

The free air in the soil.

For the first examination of the free air of the soil we have to turn, as in many other agricultural studies, to the papers of Boussingault. In 1853 he published[1] the results of analyses of 36 samples of soil gas taken at a depth of 30–40 cms. At that time Bunsen's classical memoir had not been published nor had gas analysis methods been worked out, so that he was compelled to fix a pipe in the soil (thus causing considerable disturbance) and periodically to aspirate a large volume (2½ to 10 litres) of soil air through baryta water and weigh the carbonate formed. The method must have been cumbersome to work; nevertheless the results are fairly close to ours, the air obtained from soils

[1] Boussingault and Lévy, 'Mémoire sur la composition de l'air confiné dans la terre végétale,' *Annales de Chimie et de Physique*, 1853, **37**, 5–50.

that had not recently been manured having the following mean composition:

Carbon dioxide	0·9	per cent.	by	volume
Oxygen	19·6	,,	,,	,,
Nitrogen	79·5	,,	,,	,,

It is clear that the method gives rather high results for carbon dioxide because atmospheric air was found to contain 0·04 per cent. instead of 0·03 per cent. The air from a recently manured soil contained much more carbon dioxide—up to 10 per cent.—while the oxygen fell as low as 10 per cent.[1]: but as these are the only two out of the 36 they have been omitted from the general mean.

Boussingault and Lévy did not continue their analyses over any prolonged period, nor did they study the effect of conditions such as temperature, moisture content, etc., on the composition of the soil atmosphere. These problems were investigated in Germany and the work was the outcome of the discovery by Pettenkofer[2] of a simple and rapid method of estimating carbon dioxide which he successfully applied in determining the amount of carbon dioxide in the air of the Munich soils[3]. This new method was much more rapid than the older one of Boussingault, enabling many determinations to be made and not requiring great skill in manipulation. Hence a number of workers took it up and a succession of papers on the subject appeared in Wollny's Journal[4] also published from Munich.

It is unnecessary to review all the papers in detail: especially as this has already been done by Fodor[5], Wollny[6], and Letts and Blake[7]. Moreover, later work has shown that the results are about 30 per cent. too high[8]. For comparative purposes, however, the method serves

[1] We cannot help thinking there must have been some mistake here; in our experience the oxygen falls very low only in waterlogged soils (p. 32).

[2] Letts and Blake (*Proc. Roy. Soc. Dublin*, 1900, **9**, 116) have shown that the principle of the method had already been used by Dalton and his pupils, but this work seems to have been unknown to Pettenkofer.

[3] M. von Pettenkofer, 'Ueber den Kohlensäuregehalt der Grundluft im Geröllboden von München in verschiedenen Tiefen und zu verschiedenen Zeiten,' *Zeitsch. f. Biologie*, 1871, **7**, 395–417; and 1873, **9**, 250–257.

[4] *Forschungen auf dem Gebiete der Agrikultur-Physik*, 1878–1898.

[5] J. Fodor, *Hygienische Untersuchungen über Luft, Boden und Wasser*, Braunschweig, 1881.

[6] E. Wollny, *Die Zersetzung der organischen Stoffe*, 1897.

[7] E. A. Letts and R. F. Blake, 'The carbonic anhydride of the atmosphere,' *Proc. Roy. Soc. Dublin* 1900, **9**, 107–270, especially pp. 214 *et seq.*

[8] Caldwell, in Letts and Blake's paper, *Proc. Roy. Soc. Dublin* 1900, **9**, 219–220.

sufficiently well. Successive workers showed that the amount of carbon dioxide in the soil air increased with the amount of organic matter, the water content, and the temperature of the soil. On one point, however, there was considerable disagreement which has survived to our own day: the effect of a growing crop on the production of carbon dioxide in the soil. F. Ebermayer[1] found less carbon dioxide in the soil of a wood than in a fallow soil. Möller[2] in one experiment found more carbon dioxide when a crop of grass was growing, in another less, but the conditions were not strictly comparable. In a better experiment Wollny[3] found that the effect depended on the season: in summer the cropped land (grass) was poorer in carbon dioxide than the fallow land while in winter it was richer. Of the various papers published during this early period this one by Wollny is of rather special interest because it contains numerous CO_2 values obtained between May and September which show an early summer minimum and late summer (end of August) maximum just like ours do. Numerous determinations were also made by Fodor at depths of 1, 2 and 4 metres below the surface of the soil and these showed a maximum percentage of CO_2 in July and a minimum in January or March[4]. No spring maximum was observed.

The earlier workers ascribed the formation of carbon dioxide to the decomposition of the organic matter and generally assumed that the process was the purely chemical "eremacausis" pictured by Liebig. But it was gradually recognised that soil contained numbers of micro-organisms and in 1880 Wollny[5] adopting the method of Schloesing and

[1] Ebermayer, 'Mitteilungen über den Kohlensäuregehalt der Waldluft und des Waldbodens im Vergleich zu einer nicht bewaldeten Fläche,' *Forsch. auf dem Gebiete der Agrik.-Physik*, 1878, **1**, 158–161.

[2] Joseph Möller, 'Ueber die freie Kohlensäure im Boden,' *ibid.* 1879, **2**, 329–338.

[3] E. Wollny, 'Untersuchungen über den Einfluss der Pflanzendecke und der Beschattung auf dem Kohlensäuregehalt der Bodenluft,' *ibid.* 1880, **3**, 1–15.

[4] Fodor, *loc. cit.* pp. 125 *et seq.*

[5] 'Untersuchungen über den Kohlensäuregehalt der Bodenluft,' *Landw. Versuchs. Stat.* 1880, **25**, 373–391.

An earlier reference to the possible significance of microorganisms in producing the carbon dioxide of the soil occurs in a paper by Joseph Möller, 'Ueber die freie Kohlensäure im Boden' (*Mitt. aus dem forstlichen Versuchswesen Oesterreichs*, 1878, Heft. 2, 121–148). After showing that the amount of carbon dioxide is increased by additions of organic matter he goes on to state that the lower organisms and organic residues brought in from the air are of considerable importance in this connection.

We have been unable to see the original paper, but in the long abstract in Wollny's *Forschungen* no reference is made to any experiments and it does not appear that this was more than an expression of opinion. At any rate it made no impression and it is not referred to by other writers, nor even by Möller himself in his second paper already quoted.

Müntz demonstrated that these were the active agents, the proof being that, in presence of chloroform, soil produces only a fraction of the amount of carbon dioxide formed in untreated soil. This was confirmed by Déherain and Demoussy[1]. From that time it has been generally recognised that the carbon dioxide is mainly produced by the organisms of the soil.

The application of the Pettenkofer method had thus carried the problem a long way, and had given considerable information about the origin and fluctuations of the carbon dioxide in the soil air, but it gave no information at all about the oxygen, and the idea gradually became fixed that the soil atmosphere was deficient in oxygen, a view that was strengthened by the well-known benefits of "aerating" the soil.

Boussingault and Lévy had indeed shown that the percentage of oxygen in the soil air was almost the same as that in the atmosphere, but their results were overlooked. As a matter of fact they rather contributed to the growth of the idea, for in their paper they laid chief stress on the fact that soil air contained 22 times as much carbon dioxide as ordinary air, and did not emphasise its close similarity in oxygen content.

With the introduction of improved methods of gas analysis it became possible to obtain still further refinements in the study of the soil atmosphere. Schloesing *fils*[2] was one of the first to apply the new methods and although his investigation was not very extensive it sufficed to demonstrate the incorrectness of the current conception that the soil air was necessarily deficient in oxygen.

In 1880 Hempel published his book describing a fairly accurate form of gas analysis apparatus which is as easy to use as Pettenkofer's and readily allows of the examination of large numbers of samples of air taken from the soil. It was adopted by Erich Lau in a series of analyses of the air from the soil at Rostock[3], one sample a month being taken from a sand, a loam, and a peat soil. The general result is that the soil air closely resembles ordinary air in its oxygen content, but that it contains about six times as much carbon dioxide; the actual mean values obtained at a depth of 15 cm. were, in percentages by volume:

[1] *Ann. Agron.* **22**, 305.

[2] Th. Schloesing *fils*, 'Sur l'atmosphère confinée dans le sol,' *Compt. Rend.* 1889, **109**, 618–20, 673–76.

[3] Erich Lau, *Beiträge zur Kenntnis der Zusammensetzung der im Ackerboden befindlichen Luft*, Inaug. Dissertation, Rostock, 1906.

	Sand	Loam	Peat	Sandy soil, dunged	
				Cropped with potatoes	Fallow
Carbon dioxide ..	0·11	0·14	0·43	0·57	0·18
Oxygen	20·79	20·69	20·35	20·22	20·73
Nitrogen	79·10	79·17	79·22	79·21	79·29

The minimum amounts of carbon dioxide (0·04, 0·05 and 0·12 per cent. in the sand, loam, and peat respectively) were found in February, the maximum (0·18, 0·31, and 0·81 per cent.) in July and August: no spring maximum was observed, but this might easily have been missed in the five weeks that elapsed between the taking of the May and the June samples. Some of the plots were planted and some not: the former contained more carbon dioxide than the latter, even in the summer; a result directly opposite to that obtained by Wollny.

Jodidi and Wells adopted Orsat's simpler form of the apparatus, and made a great number of analyses of the soil air from certain plots at Ames, Iowa, over the period April to August, 1910. The mean of all the results showed that at a depth of 7 inches the percentage of oxygen is 20·51, of carbon dioxide 0·25, and of nitrogen 79·24.

These various results are set out in Table IV and taken in conjunction with our own (Table VI) they establish beyond any reasonable doubt the close similarity between the soil air and the atmospheric air so far as oxygen and nitrogen content are concerned.

Table IV. *Mean composition of soil air.*

Percentage by volume of:			Locality	Investigators	Date
Oxygen	Nitrogen	Carbon dioxide			
20·6±0·2	79·2±0·2	0·2±0·1	Rostock, Germany	Erich Lau	1906
20·4±0·2	79·4±0·2	0·2±0·2	Ames, Iowa	Jodidi and Wells	1911
20·6±0·2	79·2±0·2	0·25±0·1	Rothamsted	Appleyard and Russell	1913–14

These figures are the means of the averages of the various plots.

The significance of the fluctuations in composition in the soil air.

As already stated the composition of the soil air at any moment is a resultant effect, being the difference between the rate at which the carbon dioxide is produced in the soil and that at which it is lost. At first sight it might appear that the composition must therefore be largely accidental but we have been able to show that it is not, and that the great fluctuations as distinct from the minor ones (p. 33) are regulated mainly by the rate of production of carbon dioxide in the soil. The method consists in finding some other substance in the soil which is *produced* in the same manner as the CO_2, but *lost* in a different way. If the curve showing the fluctuations of this substance is like the curve for CO_2 it follows that the fluctuations are largely governed by the rate of production and therefore that the curves given in Figs. 1–5 are essentially production curves. If on the other hand the fluctuations do not resemble those of CO_2 it follows that the curves are not essentially production curves but that their shape is due to a fortuitous balance of losses and gains.

The required substance is found in the nitrates of the soil which, like the carbon dioxide, are produced in the decomposition of the soil organic matter by bacteria but which are lost in a wholly different manner. Carbon dioxide is lost by gaseous diffusion, a process which proceeds most rapidly in dry conditions when the pores of the soil are most widely open: and least rapidly in wet conditions when the pores are more or less closed. The nitrates, on the other hand, suffer least loss under dry conditions and most loss in wet weather.

Determinations were therefore made of the amount of nitrate present in each plot on every occasion when samples of gas were drawn for analysis, and the values are plotted in the curves: unfortunately the necessity for this was not seen when the investigation first began so that no values were obtained during the first four months.

Inspection of the curves shows that they are all of the same type: there is some displacement in point of time but no difference in character. It follows then that the character of the fluctuations of CO_2 content in the soil air is determined by the rate of biochemical change in the soil. Further proof is afforded by the fact that the curves for bacterial numbers also show a close resemblance to those of CO_2 in the soil air.

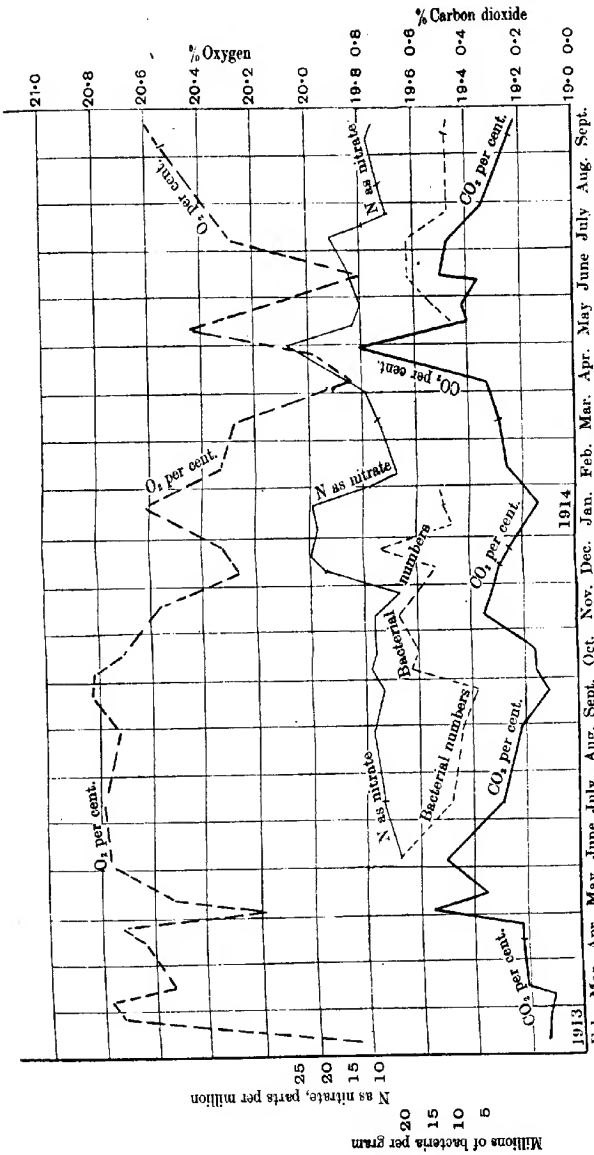
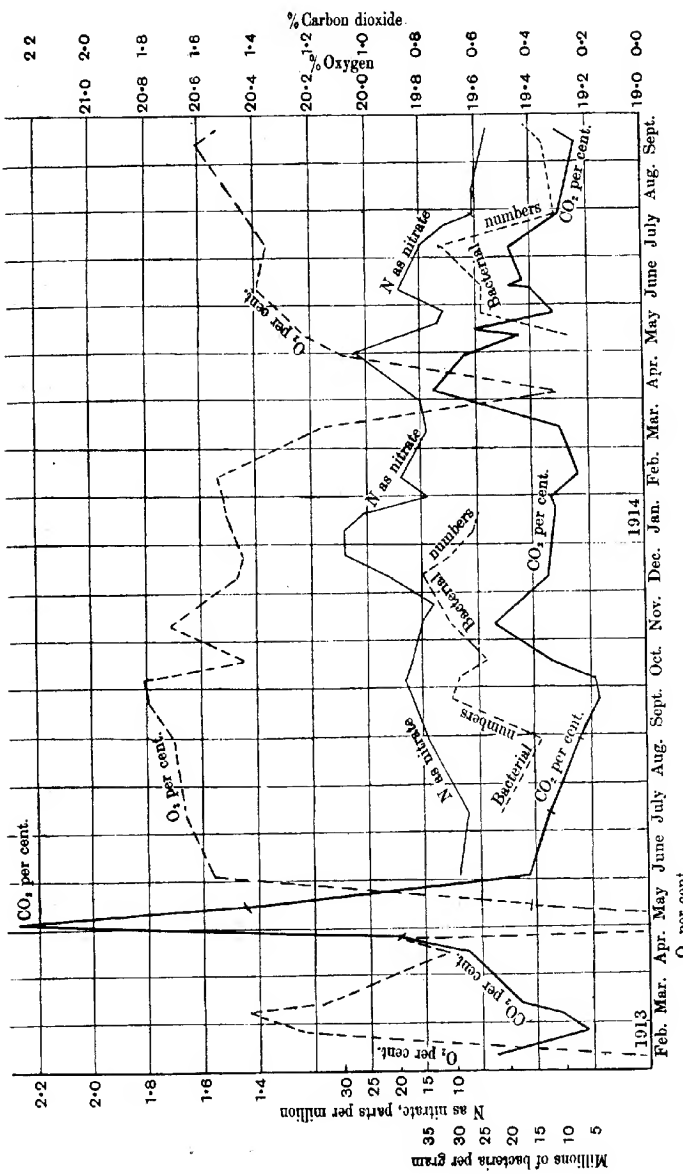


Fig. 1. Curves showing percentage of CO_2 and O_2 in soil air and bacterial numbers (millions per gram). and nitrate (parts per million) in Broadbalk unmanured plot.


% Carbon dioxide
% Oxygen
CO₂ per cent.
O₂ per cent.
N as nitrate
Bacterial numbers
CO₂ per cent.
1913
1914
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept.
N as nitrate, parts per million
Millions of bacteria per gram
O₂ per cent. falls to 17·6

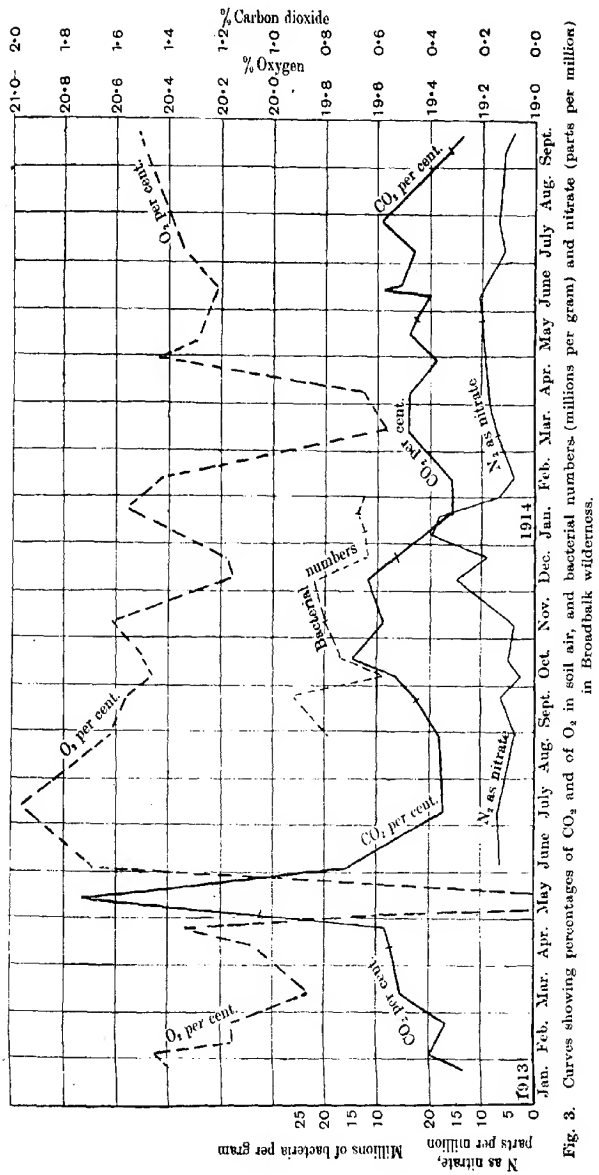


Fig. 3. Curves showing percentages of CO_2 and of O_2 in soil air, and bacterial numbers (millions per gram) and nitrate (parts per million) in Broadbalk wilderness.

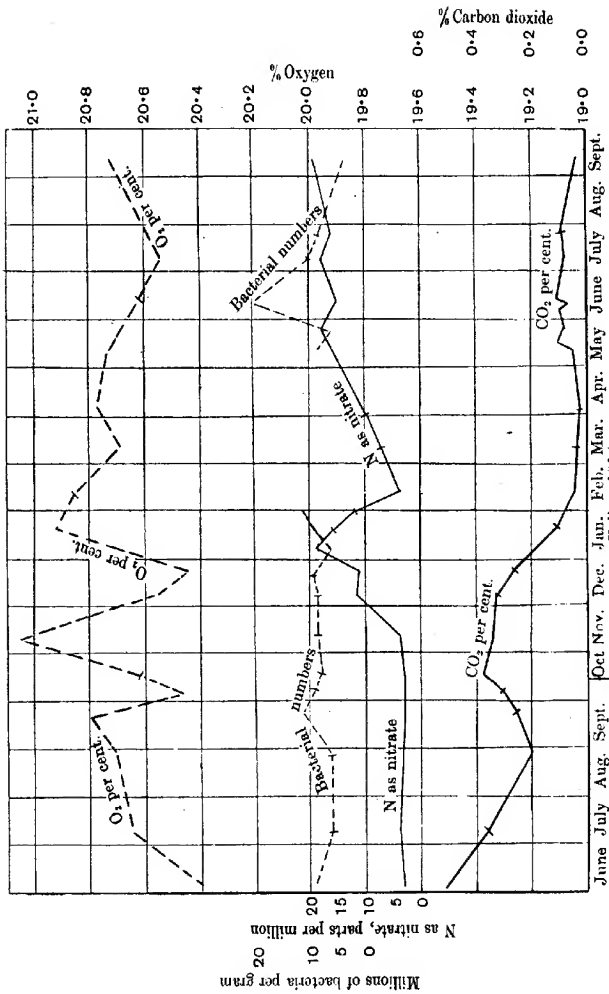


Fig. 4. Curves showing percentages of CO_2 and O_2 in soil air of Hoos wheat and fallow plots.
(*a*) Cropped till Sept. 1913, Fallow during season 1913–14.

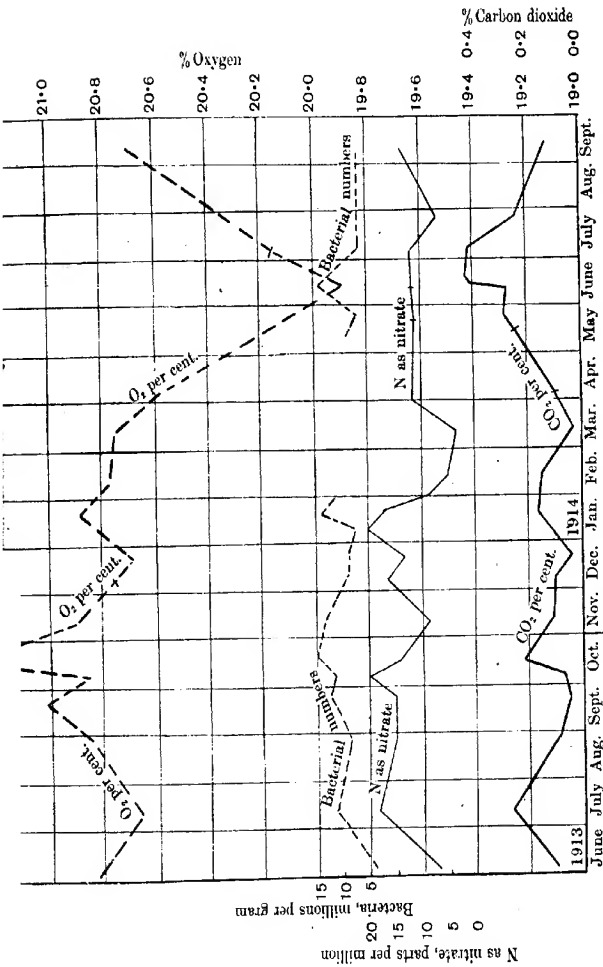


Fig. 5. Curves showing percentages of CO_2 and of O_2 in soil air of Hoos wheat and fallow plots. (*b*) Fallow till Oct. 1913, cropped during season 1913–14.

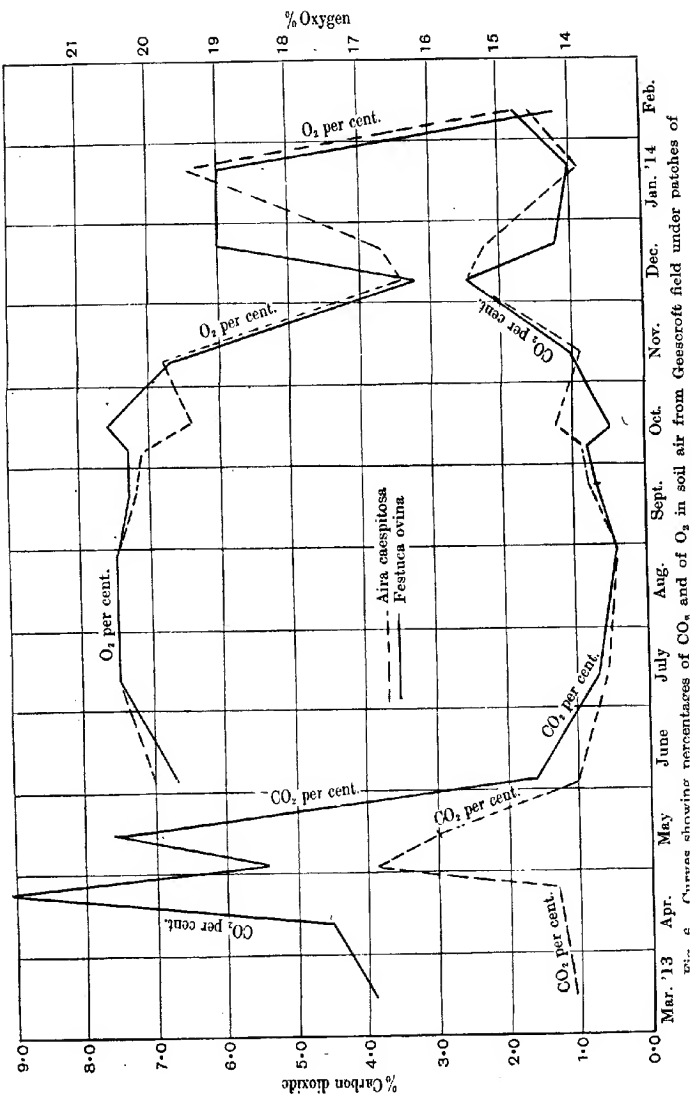


Fig. 2. Curves showing percentages of CO_2 and of O_2 in soil air from Geescroft field under patches of

The relationship of CO_2 to oxygen.

The oxygen curves are generally reciprocal to the CO_2 curves, *i.e.* the oxygen falls as the CO_2 rises, and the agreement is sufficiently close to justify the assumption that the oxygen is mainly used up in producing CO_2. But the agreement is not absolute and the discrepancies are considerably beyond the limits of experimental error.

TABLE V. *Relationship of CO_2 to oxygen at times of rapid nitrification.*

Plot	Period	CO_2 in soil air %	O_2 in soil air %	Sum	Fall in O_2 in excess of rise in CO_2	Increase in nitrate during period, parts of N per million
Broadbalk dunged	Nov. 1913	0·54	20·72	21·26		
	Dec. „	0·35	20·47	20·82	0·44	7
	Mar. 1914	0·30	20·16	20·46		
	April „	0·76	19·31	20·07	0·39	13
	May „	0·44	20·22	20·66		
	June „	0·43	20·39	20·82	−0·16	7
Broadbalk wilderness	Nov. 1913	0·58	20·62	21·20		
	Dec. „	0·64	20·17	20·81	0·39	11
	Dec. „	0·53	20·19	20·72		
	Jan. 1914	0·32	20·55	20·87	−0·15	9
Broadbalk unmanured	Nov. 1913	0·35	20·56	20·91		
	Dec. „	0·29	20·27	20·56	0·35	10
	Mar. 1914	0·29	20·28	20·57		
	April „	0·34	19·85	20·19	0·38	17
Hoos fallow	Nov. 1913	0·33	21·06	21·39		
	Dec. „	0·32	20·57	20·89	0·50	8
	Feb. 1914	0·04	20·85	20·89		
	May „	0·05	20·73	20·78	0·11	11
Hoos wheat	Nov. 1913	0·11	20·90	21·01		
	Dec. „	0·10	20·76	20·86	0·15	8
	Mar. 1914	0·03	20·75	20·78		
	April „	0·10	20·58	20·68	0·10	7

At least two cases occur in which the oxygen decreases to a greater extent than the CO_2 increases:

(1) At times of active nitrification.

(2) After heavy rainfall.

In the first case the falling off of oxygen is partly at any rate the result of oxidations such as the production of nitrate which do not yield a volume of CO_2 equal to that of the oxygen absorbed. Table V gives the results obtained for all the periods of rapid nitrate accumulation: in all except two the fall in oxygen is greater than the rise of CO_2.

The second case is seen in wet weather particularly in February, 1913 and 1914, but it reaches its maximum development on Geescroft during the period when the soil lies waterlogged; the oxygen then falls as low as 2·6 per cent. but the CO_2 does not rise above 9·1 per cent. There is no evidence of rapid biochemical change; it appears more probable that the CO_2 is being dissolved in the soil water.

There are still other instances where the fall in oxygen precedes the rise in CO_2: these are readily seen by inspecting the curves.

A third case presents more difficulty and has not yet been satisfactorily explained. Reference to the figures shows that several periods occur when the oxygen and CO_2 rise simultaneously: such are May–June 1913 and April 1914 on Broadbalk unmanured plot (Fig. 1), February, April and November 1913 on Broadbalk dunged plot (Fig. 2), March, April and October 1913 on Broadbalk wilderness (Fig. 3), etc. The phenomena suggest an evolution of CO_2 from the water or colloids in the soil.

In general the oxygen falls below that present in atmospheric air (20·97 per cent.) but in a few cases it exceeds this amount[1]. The occurrence is so rare that we have been unable to make a satisfactory investigation, but we incline to the view that the additional oxygen comes dissolved in the rain (p. 23). The following are instances:

		% CO_2	% O_2	% N_2
Hoos field wheat	10 Nov. 1913	0·69	21·01	78·30
		0·10	21·10	78·78
		0·19	21·71	78·10
Broadbalk wheat (dunged plot)	10 Nov. 1913	0·11	21·19	78·70
Geescroft	10 Nov. 1913	0·19	21·21	78·60

[1] See also Appendix, Table XI, Hoos field fallow.

THE CAUSES OF FLUCTUATIONS OF COMPOSITION OF SOIL AIR.

A. *The variations due to season.*

These fluctuations consist in a rise to a maximum CO_2 content in late spring, a fall to a minimum in summer, a rise to a second maximum in late autumn and a fall to a minimum in winter. The oxygen content varies in the inverse sense, reaching minimum values in spring and autumn and maximum values in summer and winter.

All the curves show the same general shape when plotted over the year; proving that the effect of season completely overrides the effect of various soil treatments. Field experiments alone do not enable us to disentangle all the factors, but we took measurements for the purpose of discussing the effect of temperature and moisture content.

Effect of temperature. This can be studied from Fig. 7 where the mean soil temperatures taken from the continuous recording soil thermometer are plotted along with the CO_2 in the soil air from the Broadbalk unmanured plot.

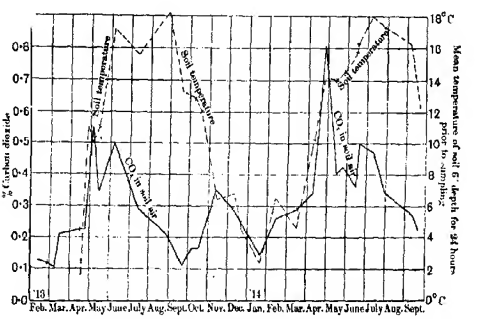


Fig. 7. Curves showing percentage of CO_2 in air of Broadbalk unmanured plot and mean soil temperature (at 6″ depth) for 24 hours previous to sampling.

Beginning with the middle of April, 1913, when soil temperatures were first taken, it is seen that the temperature curve runs closely with the CO_2 curve up to the early part of May, they then part company and show no more resemblance till November. From that time, however, up to early May, there is a close general resemblance but this ceases from then onwards. Thus we can infer that the temperature is the dominating factor in determining the amounts of CO_2 production from November to May.

It is clearly not the only factor for the parallelism is not complete: a rise in temperature in spring is more potent to increase the output of CO_2 than a similar rise later on. Thus the values for temperature and CO_2 in May and June no longer show the agreement obtained earlier: the CO_2 maximum in May being above that in June while the temperature maxima fall the other way. These differences in detail indicate that other factors are operating, but they do not weaken the main conclusion that *from November to May the temperature determines the rate of* CO_2 *production in the soil*[1].

The dunged plots and the wilderness show the same general relationships, but again there are differences in detail, the CO_2 and temperature curves parting company earlier in the summer than on the unmanured plot. The main obvious difference between the plots is that the crop is larger on the dunged plot and the wilderness than on the unmanured plot, and the bearing of this factor will become evident later on.

From June to November, however, the temperature is not the main factor for the curves show no kind of similarity.

Effect of Moisture. A comparison of moisture content and CO_2 content is made in Fig. 8. The moisture determinations only began in June 1913, so that the curve does not run as long as that for temperature but it shows no connection with the CO_2 curves except during a few months in summer. The moisture is low during June, July and August of 1913 when the CO_2 is falling: it rises in September and October when the CO_2 first falls and then rises, it is steadily high from November to March 1914 during which the CO_2 first falls and then rises; it falls in April while the CO_2 rises and falls low during summer when the CO_2 also is low.

Thus moisture does not have nearly so marked an effect as temperature, and it only shows any relationship to the CO_2 during the summer months July to September.

The extreme case of water logged soil is dealt with on p. 32.

[1] The failure to find on some of the plots a maximum CO_2 content in May 1914 of the same order as the value obtained in 1913 may be attributed to the fact that quite unwittingly we allowed a favourable temperature period to pass without taking any samples. We made determinations on May 15 and again on May 25, but during the interval there came a rise in temperature which we missed.

May 1914	15th	16th	17th	18th	19th	20th	21st	22nd	23rd	24th
Soil temperature at depths of 6 in. °C.	15·5	15·1	16·1	17·3	16·9	17·9	19·0	20·0	16·1	14·6

Rainfall. If instead of taking the percentage of moisture, we plot rainfall for the week preceding the date of sampling, we obtain a somewhat closer relationship with the CO_2 curves (Fig. 9). The May maximum (1913) is seen to coincide with a period of high rainfall: the

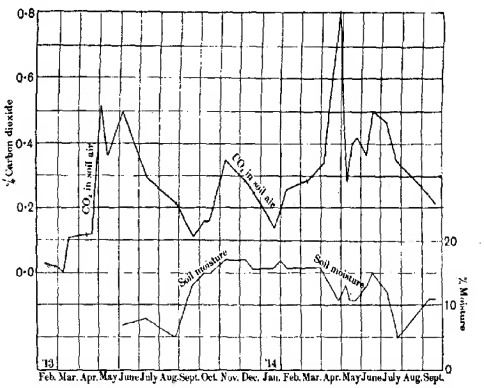


Fig. 8. Curves showing percentage of CO_2 in air of Broadbalk unmanured plot and soil moisture to a depth of 9″.

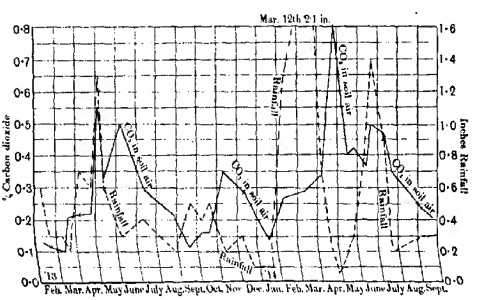


Fig. 9. Curves showing percentage of CO_2 in air of Broadbalk unmanured plot, and rainfall for seven days preceding day of sampling.

October maximum follows after a second high rainfall and the intervening summer minimum is in a dry period: the April (1914) and the June maxima occur with other high rainfall periods. These are not simple moisture effects, for they are not brought out so clearly on the moisture curve, and we have to seek some other explanation. Two factors appear

to come into play. In the first place the rain does not immediately distribute itself throughout the soil but produces a more or less saturated layer which seals the surface and prevents the escape of CO_2 from the soil air. Further, rain appears to be nearly saturated with dissolved oxygen. We have already seen that the dissolved atmosphere in the soil tends to lose oxygen more rapidly than to gain it and in consequence is largely anaerobic. A large fall of rain bringing with it oxygen in solution affords the possibility of partially renewing the dissolved atmosphere and giving the organisms a new lease of activity. In time, however, the oxygen is used up and the activity falls off even though the moisture remains constant. This effect is probably most marked when the soil is dry and the new dissolved atmosphere can most completely replace the old one. We could find no determinations of the amount of dissolved oxygen in rain water but a number of analyses of stream waters have been made by the Sewage Commission, and they show that on an average about ten parts per million by weight of dissolved oxygen is present. If we suppose that rain contains approximately the same amount then 1 inch of rain brings down $2\frac{1}{4}$ lbs. of oxygen per acre; this if converted into CO_2 would add 0·8 to the normal 0·2 per cent. by volume and make the total up to 1 per cent. In addition the rain itself brings down a certain amount of CO_2, but not much, and considerably less than the amount of oxygen.

Relation between soil air and atmospheric air. The experiments described in this section show that CO_2 is produced at maximum rates in spring and in autumn and at minimum rates in summer and winter. As it is constantly escaping from the soil into the atmosphere we should naturally expect to find that the CO_2 in the atmospheric air also reaches maximum amounts in spring and autumn, minimum amounts in summer and winter.

Systematic determinations of the amount of CO_2 in atmospheric air are not numerous, but those made prior to 1899 were collected by Letts and Blake in their paper already quoted[1]. A statistical examination of the data shows that, as far as they can be relied upon, they indicate an increase in atmospheric CO_2 during the period March–May, a falling off during the period May to August, and a rise during the period October to January. Thus a very close agreement is obtained with our soil results.

[1] *Proc. Roy. Soc. Dublin*, 1900, **9**, 107–270 and especially pp. 205 *et seq.*

B. *The effect of organic matter.*

Fig. 10 shows the comparison between two plots in Broadbalk wheat field one of which is unmanured while the other receives every September a dressing of 14 tons of farmyard manure. The comparison is only strict during the winter period September to March or April when the

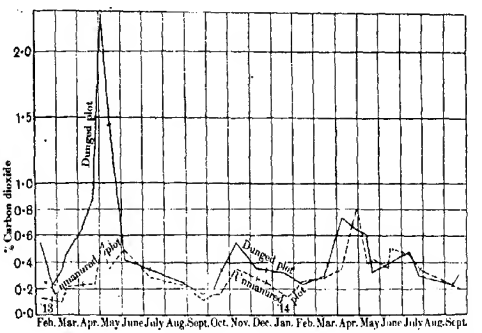


Fig. 10. Comparison of CO_2 content of unmanured plot with plot receiving farmyard manure, Broadbalk field.

crop is so small that it can safely be neglected; from May on to harvest time complication arises from the fact that the dunged plot carries a dense crop while the unmanured plot does not. During winter the air from the dunged plot consistently contains the larger quantity of CO_2; we can carry the strict comparison from March onwards by taking the fallow part of the dunged plot and the unmanured fallow on Hoos field, which closely resembles the unmanured plot in Broadbalk:

	May 15	May 25	June 10	June 12	June 13	July 7	July 27
Dunged fallow (Broadbalk) ..	0·22	0·32	0·17	0·36	0·36	0·36	0·35
Unmanured fallow (Hoos)	0·10	0·07	0·08	0·07	0·10	0·08	0·09

The dunged plot still gives the higher result so that the effect of the manure is clearly to increase the amount of CO_2 in the soil air throughout the year.

The persistence of this increase is its chief characteristic, and during most of the year it does not assume very great dimensions nor does it alter the shape of the curve relative to the unmanured land. The actual percentages of CO_2 during the month before and the month after ploughing in are as follows:

	Dunged plot before ploughing in	Unmanured plot		Dunged plot after ploughing in	Unmanured plot
September 22	0·17	0·11	November 10	0·54	0·35
October 6	0·18	0·16	December 9	0·35	0·29
„ 17	0·34	0·16	„ 12	0·34	0·25

Considerably larger differences however were observed during the spring both in CO_2 and oxygen in 1913 and in oxygen in 1914.

C. *The effect of a growing crop.*

As already pointed out (p. 9) there has been considerable disagreement as to the relative amounts of CO_2 in the air of cropped and of uncropped soils. Critical examination of the older work shows that much of the discussion was irrelevant because the conditions in the various experiments were not comparable. A cropped plot differs in physical state, moisture content, temperature, etc. from uncropped land and when the case is pushed to an extreme and a comparison is instituted between grass land and arable land there arises a further complication due to the difference in organic matter content of the two soils.

The usual method has been to set up a comparison between cropped and fallow portions of the same plot. We have done this in two fields. Figs. 4 and 5 and Table VI give the detailed results and Fig. 11 a simpler comparison for the Hoos wheat and fallow plots. These are made to alternate each year: the land has been unmanured since 1851 and now yields a small crop averaging 16 bushels of wheat per acre. All through the period of active growth (June to August) the cropped plot is the richer in CO_2 and it maintains its superiority even after the crop is cut and right up to the time when the land is ploughed. Then the CO_2 sinks to a low level and remains low throughout the period of fallow; it rises again as soon as the land comes into crop. The physical differences in the plots, however, are considerable. The fallow land is left rough and is not harrowed, it is occasionally cultivated to kill weeds, thus it readily allows of the escape of CO_2. The cropped land has to

be got into a tilth for the seeding and it speedily becomes compact and less favourable to gaseous diffusion.

During the current year the top half of Broadbalk field has been fallowed and a comparison was made between the fallow and the

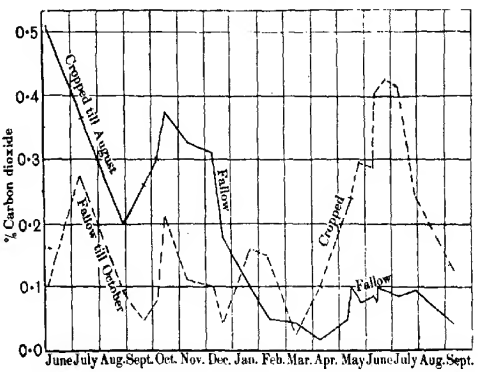


Fig. 11. Curves showing percentage of CO_2 in air of Hoos wheat and fallow plots.

cropped portions of the dunged plot. Here the conditions are different from those in Hoos field; the soil contains considerably more organic matter and does not become very compact: the difference in physical condition between the cropped and fallow portions therefore is not nearly so marked (although it still exists) and a stricter comparison is possible. Moreover the crop (which was fairly dense) did not apparently affect the temperature of the soil, and from May to July practically no differences were observed[1]. The moisture content, however, was affected, the percentage of water being:

	June 12	July 7	July 27	
Fallow portion	18	20	12	per cent. of water
Cropped portion	19	17	9	

[1] The actual readings (6″ depth) were:

	May 15	May 25	June 10	June 12	June 13	July 7	July 27
Fallow portion ..	12°	11°	12°	12°	14°	15°	15°
Cropped portion ..	12°	11°	12°	12°	14°	15°	14°

Thus the soil conditions are still not entirely comparable but on the whole they are more so than on Hoos field. The percentages of CO_2 in the soil air were:

	May 15	May 25	June 10	June 12	June 13	July 7	July 27
Fallow portion	0·22	0·32	0·17	0·36	0·36	0·36	0·35
Cropped portion	0·61	0·32	0·35	0·48	0·42	0·48	0·30

Now the crop was considerable (30·4 bushels per acre), yet the increase in CO_2 over that in the fallow plot is not only no greater than in Hoos field but it is not usually (except on May 15) much larger than the error of experiment. Hence it appears that the effect of the growing crop in increasing the amount of CO_2 in the soil air is not great.

We can make the comparison in a different way so as to reduce in another direction the differences in physical state between the plots. The Broadbalk dunged and unmanured cropped plots both receive similar cultivations and treatment apart from manuring: both are equally exposed to the consolidating effect of the weather though the unmanured land does actually become the more closely packed. The dunged land possesses a large quantity of organic matter and carries a dense crop, both conditions favourable for a high percentage of CO_2 in soil air, yet as a matter of fact this high percentage is not obtained, and in summer when one would expect the maximum differences from the unmanured plot there is practically no difference at all[1].

[1] On the following occasions the unmanured plot gave a higher CO_2 content than the dunged plot in Broadbalk field:

	Date	Mean composition of soil air		Moisture	Temperature °C.	
		% CO_2	% O_2	Per cent. in soil	Air	Soil
Unmanured plot	3 June 1913	0·50	20·77	7	22	18
Dunged plot		0·42	20·56	11		15
Unmanured plot	29 April 1914	0·81	19·98	11	10	—
Dunged plot		0·65	20·08	16		—
Unmanured plot	25 May 1914	0·42	—	11	10	12
Dunged plot		0·32	—	13		11
Unmanured plot	13 June 1914	0·50	19·80	15	21	15
Dunged plot		0·42	20·38	19		14
Unmanured plot	27 July 1914	0·35	—	5	14	16
Dunged plot		0·30	—	9		14

Determinations of the amount of CO_2 in the soil air of grass land are given in Table VII. The results show that more CO_2 is usually present than in arable land and the oxygen content is lower. But no strict comparison with arable land can be made because of the great

TABLE VII. *Composition of soil air of grassland. Percentage by volume.*

A. *Pasture used for grazing.*

Date	CO_2	O_2	N_2
Nov. 6, 1912	1·01	18·72	80·27
„ 14 „	1·59	18·12	80·29
„ 20 „	1·99	—	—
„ 21 „	1·35	—	—
„ 22 „	1·90	—	—
Dec. 2, 1913	3·34	15·18	71·48
Jan. 30, 1914	1·46	18·44	80·10
Jan. 30, 1914, 18 in. deep	1·64	17·87	80·49

B. *Geescroft Wilderness.*

Date	CO_2	O_2	N_2	CO_2	O_2	N_2	Bacterial numbers, millions per gram	N as nitrate, parts per million
Dec. 19, 1912	1·5	15·8	82·7					
Jan. 13, 1913	0·7	16·6	82·7					
Jan. 24 „	3·1	6·2	90·7					
Feb. 11 „	0·7	19·0	80·3					
Feb. 26 „	2·0	16·4	81·6					
	under *Festuca ovina*			under *Aira caespitosa*				
Mar. 13, 1913	3·9	13·0	83·1	1·1	19·4	79·5		
April 14 „	4·5	9·2	86·3	—	—	—		
April 24 „	9·1	2·6	88·3	1·3	19·1	79·6		
May 2 „	5·4	9·0	85·6	3·9	10·6	85·5		
May 13 „	7·6	8·6	83·8	3·0	14·5	82·5		
June 3 „	1·6	19·7	78·7	1·0	20·0	79·0	8	3
July 11 „	0·7	20·5	78·8	0·6	20·5	78·0	8	4
Aug. 29 „	0·4	20·5	79·1	0·4	20·5	79·1	9	4
Sept. 22 „	0·7	20·3	79·0	0·8	20·2	79·0	17	2
Oct. 6 „	0·8	20·3	78·9	0·9	20·1	79·0	—	5
Oct. 17 „	0·5	20·6	78·9	1·2	19·4	79·4	14	3
Nov. 10 „	1·0	19·7	79·3	0·9	19·8	79·3	10	1
Dec. 9 „	2·5	16·2	81·3	2·5	16·4	79·1	10	8
Dec. 22 „	1·2	19·0	79·7	2·2	16·7	81·1	17	6
Jan. 8, 1914	—	—	—	—	—	—	13	—
Jan. 20 „	1·0	19·0	80·0	0·9	19·5	79·6	8	11
Jan. 30 „	—	—	—	—	—	—	13	7
Feb. 12 „	1·8	14·2	84·0	1·6	14·8	83·6	—	6

difference in amount and composition of the organic matter present in the soil. The closest comparison we can set up is between two of the Broadbalk plots: an arable plot receiving 14 tons of dung annually and carrying each year a good crop of wheat, and an adjacent plot known as the wilderness which has remained undisturbed since 1882 and now carries a dense growth of grasses, clovers, weeds, etc., only young trees and bushes being removed. The percentages of CO_2 in the soil air are plotted in Fig. 12. There is no great difference between the two curves. In April and early May the dunged plot contains more CO_2, from September to early January it contains less, but during these months it has been ploughed up and left loosely exposed to the atmosphere for a time prior to seeding. But the differences rarely

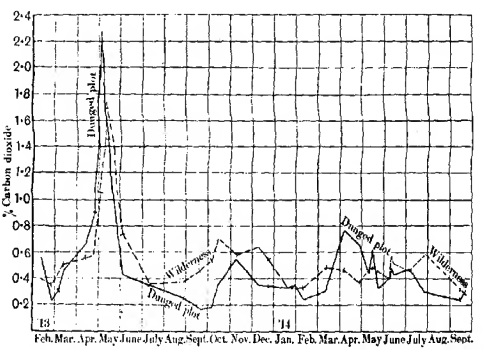


Fig. 12. Curves showing CO_2 in soil air of Broadbalk dunged and wilderness plots.

exceed 0·3 per cent. When therefore the soil conditions are comparable both as to the state of packing and to the amount of organic matter the difference between grass and arable land is less than might be expected. The result is all the more significant when it is remembered that the air of the unmanured plot is as rich in CO_2 during summer as the air of the dunged plot.

Taking them as a whole, these observations indicate that a growing crop *per se* has no very marked effect in increasing the amount of CO_2 in the soil air. Comparison is rendered difficult by the numerous differences between cropped and fallow land or between grass and arable land, which can only partially be eliminated; if an ordinary grass field is compared with an ordinary arable field considerable differences are found, but when the conditions are made more nearly

alike the effect of the crop is not very great. Absolute identity of conditions has not been attained, and we cannot yet be certain whether the small effect of the crop still observed is due to uneliminated soil differences such as the removal of water by the growing crop which thus facilitates the escape of CO_2 evolved from the plant roots; or to some direct interference of the growing crop with bacterial activity in the soil.

A wholly different argument in a previous paper[1], led to the conclusion that the growing plant interferes with bacterial activity.

Before leaving this subject attention must be directed to one interesting point in connection with the two Broadbalk plots, the dunged arable and the wilderness. The arable plot shows a persistent loss of nitrogen amounting to over 100 lbs. per acre per annum, apparently

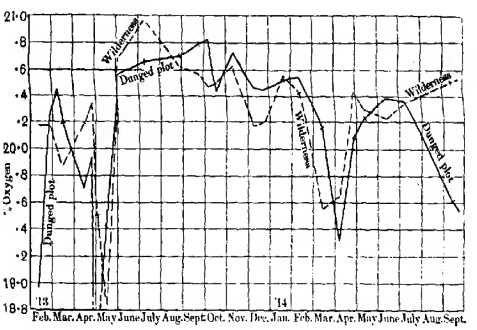


Fig. 13. Curves showing O_2 in soil air of Broadbalk dunged and wilderness plots.

not wholly by drainage. The wilderness, on the other hand, shows a persistent gain of nitrogen amounting approximately to 100 lbs. per acre per annum. We have hitherto been inclined to attribute this remarkable difference to a supposed greater aeration influencing the biochemical changes in the arable land. It is therefore of special interest to compare the oxygen content of the air from the two plots: this has been done in Fig. 13, from which it appears that there is little if any difference between them.

Amount of CO_2 *under plants of various species.* On some of the Rothamsted grass plots and especially those that have become acid there is a tendency for certain species to segregate; determinations were therefore made of the percentage of CO_2 in the soil air of these

[1] 'The nature and amount of the fluctuations in nitrate contents of arable soils,' *J. Agric. Sci.* 1914, **6**, 18–57.

various patches. It is found that there is a perceptible falling off of oxygen and rise in CO_2 in passing from a neutral matrix to a "sour" patch (indicated by the presence of rumex or in extreme cases by the total absence of all vegetation). But a patch of a solitary species occurring on a neutral plot such as plot 7 shows no such difference. The results are:

	Plot 7		Plot 5 N.		Plot 5 S.			Plot 4[2]		Plot 11-1	
Per cent.	Under matrix	Under spirea	Under matrix	Under dactylis	Under matrix	Under dactylis	Under dactylis and rumex	Under matrix	Under bare patch	Under matrix	Under bare patch
CO_2	1·5	1·4	1·2	1·5	1·3	1·1	2·0	2·1	1·5	1·2	2·3
Oxygen	19·3	20·0	20·0	19·7	20·0	20·1	19·5	19·0	19·5	20·1	18·5

Samples taken May 24th, 1913.

Another field where segregation occurs is Geescroft which is liable to become waterlogged in winter owing to the absence of calcium carbonate from the soil and the consequent deflocculation of the clay. During normal moist or dry conditions the soil air from the various patches is similar in composition and resembles that from the other fields. But in very wet conditions marked differences set in, the oxygen falling and the nitrogen[1] rising very considerably in amount; this happens particularly under the patches of *Festuca ovina* the roots of which form a densely matted tangle near the surface, but it is less marked under the patches of *Aira caespitosa* the roots of which form a bristly mass more readily allowing gaseous diffusion. The results are plotted in Fig. 6, they are as follows:

Wet conditions.

1913	% CO_2		% O_2		% N_2	
	Aira	Festuca	Aira	Festuca	Aira	Festuca
March 13	1·1	3·9	19·4	13·0	79·5	83·1
April 14	—	4·5	—	9·2	—	86·3
April 24	1·3	9·1	19·1	2·6	79·6	88·3
May 2	3·9	5·4	10·6	9·0	85·5	85·6
May 13	3·0	7·6	14·5	8·6	82·5	83·8

[1] Examination for hydrogen or methane has so far led to negative results.

Dry conditions

1913	% CO_2		% O_2		% N_2	
	Aira	Festuca	Aira	Festuca	Aira	Festuca
June 3	1·0	1·6	20·0	19·7	79·0	78·7
July 11	0·6	0·7	20·5	20·5	78·9	78·8
August 29	0·4	0·4	20·5	20·5	79·1	79·1
September 22	0·8	0·7	20·2	20·3	79·0	78·9
October 6	0·9	0·8	20·1	20·3	79·0	78·9

The low amount of CO_2 relative to the oxygen used up has already been discussed (p. 19).

Minor fluctuations in composition of the soil air.

We now turn to a consideration of the minor fluctuations in composition of the soil air. These differ fundamentally from the major fluctuation hitherto dealt with in as much as they are probably not associated with the production of CO_2 in the soil but only with variations in the agencies causing loss. They are brought about by two causes:

(1) Variations in the soil itself: shown in Table XI (p. 41) and discussed on p. 4.

(2) Variations in meteorological and cultivation conditions.

The only satisfactory way of dealing with the effect of meteorological conditions on the soil atmosphere is by statistical methods, but although we have many records we do not feel that they are sufficiently numerous for the purpose. We have, however, tested certain broad and obvious possibilities, the data for which are found in Table VI (p. 46).

(*a*) *Rapid change of temperature.* It has happened on a warm day preceded by a frosty night, *i.e.* where the temperature altered quickly and considerably, that the soil air approximated closely in composition to atmospheric air indicating that it had been largely replaced by atmospheric air. Instances occur on January 13th and February 26th, 1913.

(*b*) *High rainfall.* In view of the quantity of bicarbonates in drainage water it is important to ascertain whether high rainfall appreciably diminishes the amount of CO_2 in the soil air. The observations do not yield any very definite results: in some cases the immediate effect is to reduce the CO_2 but not always, while usually the subsequent

effect is to increase it (p. 23, Fig. 9). The following data serve as illustrations:

Date	June 10th	June 12th	June 13th
Rainfall of previous 24 hours ..	0·33 in.	—	0·65 in.
CO_2 per cent. in soil air:—			
Broadbalk unmanured plot ..	0·36	0·37	0·50
„ dunged plot ..	0·40	0·48	0·43
„ wilderness	0·40	0·58	0·51
Hoosfield wheat	0·28	0·41	0·43
„ fallow	0·08	0·07	0·10

These observations confirm the older results of Fodor[1].

(1) *Strong winds.* On several occasions, *e.g.* February 3rd, March 7th, 1913, samples were taken directly after a windy night but there was nothing at all to indicate that the composition of the air had been affected by the wind. A current of air passing rapidly over the soil might have been expected to draw out the soil air, but apparently it does not. Probably the force is insufficient, the layer of air in contact with the surface of the soil moves less quickly than the layers a few inches above. Moreover any removal of air by this process from the surface layers of the soil probably leads to an upward movement of air rich in CO_2 from the lower depths.

(2) *Change in barometric pressure.* Fodor[2] found that the CO_2 in soil air rose with falling barometer at three stations out of four where investigations were made. In the only continuous experiment we made we were fortunate in happening upon a time when the barometer was rapidly falling and we also obtained a rise in CO_2 during the period. But when the whole of our CO_2 figures are plotted against barometic pressures or even against changes in barometric pressure no consistent relationship can be observed such as is obtained with rainfall, temperature, etc., so that the influence of barometric pressure appears to be only minor and easily swamped by other factors.

(3) *Night and day.* Fodor[3] and Wollny[4] thought they had evidence that CO_2 streams out from the soil air at night but we can find no indication of any greater loss by night than by day. Samples drawn from the same 5 holes at consecutive 3-hour intervals over a period of

[1] Josef Fodor, *Hygienische Untersuchungen über Luft, Boden und Wasser*, Braunschweig, 1881, p. 130.

[2] Fodor, *ibid.* p. 135. [3] Fodor, *ibid.* p. 53.

[4] Wollny, *Forsch. auf dem Gebiete der Agrik.-Physik*, 1885, **8**, 417.

33 hours failed to show any systematic variation as between the day and the night. The results are given in Table VIII. The CO_2 tends to rise and the oxygen to fall from the 18th hour onwards (*i.e.* from 3.30 a.m. on the 15th) when the barometer is steadily falling, but there is no sign of any relationship with the temperature either of the air or the soil.

TABLE VIII. *Hourly fluctuations in composition of soil air, 3-hour periods over 33 consecutive hours.*

Hour	0	3	6	9	12
Time Nov. 14	A.M. 9.30	P.M. 12.30	P.M. 3.30	P.M. 6.30	P.M. 9.30
% CO_2 (mean)	0·11	0·13	0·11	0·15	0·13
% O_2 (mean)	20·69	20·82	20·65	20·70	20·61
Barometer mm.	742	746	747	748	749
Air temp. °C.	5	6	2	−1	−2
Soil temp. °C.	2	6	6	5	5

Hour	15	18	21	24	27	30	33
Time Nov. 15	A.M. 12.30	A.M. 3.30	A.M. 6.30	A.M. 9.30	P.M. 12.30	P.M. 3.30	P.M. 6.30
% CO_2 (mean)	0·13	0·13	0·14	0·16	0·16	0·19	0·18
% O_2 (mean)	20·61	20·62	20·52	20·54	20·51	20·42	20·43
Barometer mm.	747	744	738	733	731	730	729
Air temp. °C.	0	1	1	1	13	8	6
Soil temp. °C.	5	5	5	5	7	6	5

(4) *Cultivation.* We have not made systematic investigations into the effects of the various cultivation operations, but we find that ploughing usually increases the percentage of oxygen and diminishes the CO_2 in the soil air, the fall in CO_2 being particularly marked when the ploughing is done early. The details are given in Table IX, where also are set out the analytical data for the uncultivated wilderness.

The relation between carbon dioxide production, nitrate formation and bacterial numbers.

The curves showing the amounts of carbon dioxide in the soil air and of nitrate in the soil are so similar in character as to justify the view that both essentially represent the rates of formation (p. 12). Closer comparison of the curves with those for bacterial numbers

brings out several important features which we must now proceed to discuss.

Fig. 14 shows the rainfall, bacterial numbers, carbon dioxide and nitrate for the Broadbalk dunged plot, which is perhaps the most convenient for our purpose by reason of the high values it yields. Beginning in July, 1913, the bacterial numbers follow the rainfall very closely till October and less closely till January, the diminishing rainfall

TABLE IX. *Percentage composition of soil air before and after cultivation operations.*

Date	Uncultivated land Wilderness		Cultivated land: Broadbalk Dunged		Cultivated land: Broadbalk Unmanured		Hoos Fallow Wheat	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
July 11 ..	0·36	20·97	0·35	20·66	0·29	20·79	0·27	20·66
			Wheat cut		Wheat cut		Ploughed	
August 29 ..	0·37	20·62	0·24	20·70	0·22	20·73	0·09	20·84
			Ploughed and harrowed		Ploughed and harrowed			
September 22	0·46	20·57	0·17	20·79	0·11	20·83	—	—
October 6 ..	0·53	20·47	0·18	20·81	0·16	20·82	—	—
					Ploughed			
October 17 ..	0·70	20·50	0·34	20·43	0·16	20·72	0·21	21·30
			Ploughed, harrowed and drilled		Harrowed and drilled		Drilled with wheat	
November 10	0·58	20·62	0·54	20·72	0·35	20·56	0·11	20·90
December 22	—	—	—	—	—	—	0·17	20·44
							Ploughed	
January 20 ..	—	—	—	—	—	—	0·10	20·92

of July and August being accompanied by a fall in bacterial numbers, the September rain by a rapid rise, and so on. The CO_2 curves also follow in the same way but later in point of time and they are somewhat smoothed out: thus they do not show the kink in October. The nitrate curves again show the same rise but still later; in comparing them with the others, however, it must be remembered that conditions of drought which favour a decrease of bacteria through death and of CO_2 through diffusion have no effect in reducing the amounts of nitrate: thus during

July and August the nitrates increase instead of falling like the CO_2. But in November and December the nitrates rise sharply and keep high until the heavy February rains[1], when they fell to a minimum just as do the bacterial numbers and the carbon dioxide.

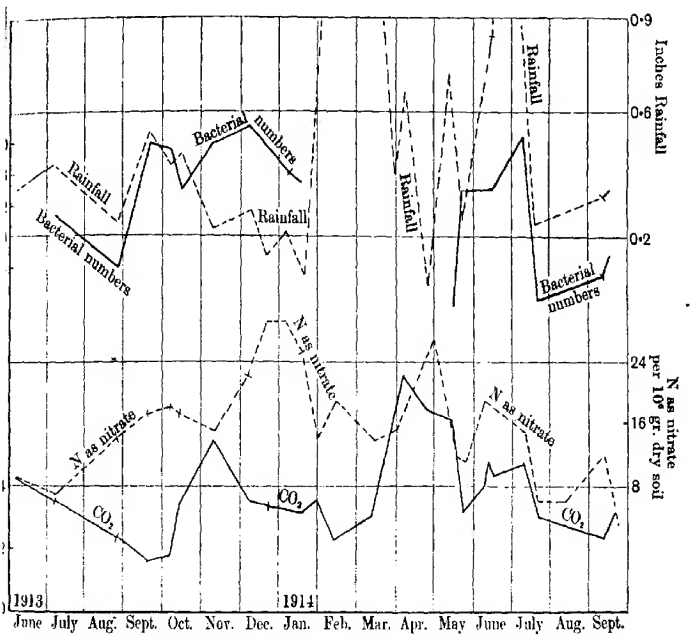


Fig. 14. Curves showing rainfall, bacterial numbers in soil, CO_2 in soil air and nitrate in soil of Broadbalk dunged plot.

[1] The rainfall for December 1913 and January 1914 was considerably below the average so that the washing out of nitrate began later than usual. The rainfall figures are:

	December	January
Average 1853–1913	2·44	2·35
This year ,,	0·88	0·88

It is interesting to note that, when the drains began to run in February 1914, the drainage water was of approximately the same order of concentration as after the similar winter conditions of 1879–80:

N as nitrate in drainage water from Plot 2 (dunged)

February 1914	26·8	29·7	parts per million
,, 1880	27·3	,,	,,

Unfortunately there was a break in the bacterial counts during the winter months, but the other observations were made. In March, 1914, there occurred a high rainfall, followed by a rise in CO_2 and somewhat later by a rise in nitrate: in April the CO_2 falls, but in May and June there is a sharp increase in rainfall and in bacterial numbers, followed by an increase of CO_2 and of nitrate.

If we take the unmanured (Fig. 1) instead of the dunged plot we obtain similar but numerically smaller results. The wilderness (Fig. 3) also shows the same general phenomena, but the spring rise in the nitrate is considerably flattened down in consequence of the rapid absorption by the plants; the autumn rise, however, is seen, and as before it comes after the rise in CO_2 and this in turn after the rise in bacteria. Again, the Hoos wheat and fallow plots (Figs. 4 and 5) show like similarity between bacterial numbers, CO_2 and nitrates, especially during the fallow period. The fluctuations are not great—the land having received no manure for many years is very impoverished—and it would be unsafe to attach too much importance to some of them, but they all go in the same direction. During the time when the land carries a crop of wheat (Fig. 5) the nitrate curve is flattened from April to July; while on the other hand the loosening of the land during the fallow period causes a flattening of the CO_2 curve.

The general conclusion is that the fluctuations in bacterial numbers, in CO_2 content and in nitrates in the soil are all of the same general character, and this character is mainly impressed by seasonal factors: other conditions such as manuring, cropping, etc., may pull out or flatten the curves but they do not alter their general shape. The production both of nitrates and of CO_2 attains a maximum in late spring or early summer, a minimum in summer, a maximum in late autumn and a minimum in winter[1]; the numbers of bacteria fluctuate in the same way in summer, autumn and winter. When the autumn rains came after the dry summer conditions, the bacteria immediately responded by rapid multiplication: then there came an increase in the amount of CO_2 in the soil air and finally an increase in the amount of nitrates. This order seems to be pretty general.

The spring and autumn periods of maximum biochemical activity in the soil are clearly of great significance in soil management.

[1] Similar seasonal fluctuations in nitrate content are recorded in the paper already quoted in *J. Agric. Sci.* 1914, **6**, 18–57.

Appendix.

I. *Method of collecting and analysing the soil air.*

The apparatus used for collecting the soil air is shown in Fig. 15; it was used by Hall and Russell in their investigations of the air of Romney marsh soils. It consists of a hollow cylindrical steel tube (*A*) 2 feet long, $\frac{5}{8}$ in. outside and $\frac{3}{8}$ in. inside diameter to which is welded a side tube (*R*) $2\frac{1}{2}$ inches from the top to allow of the air being withdrawn from the nozzle (*S*). The top of the tube is strengthened by a cap (*B*). A solid cylindrical rod (*N*) $\frac{3}{8}$ in. in diameter with a flat side $\frac{1}{8}$ in. wide running its whole length fits tightly into the hollow tube; it is provided at the bottom with a collar $\frac{1}{8}$ in. wide.

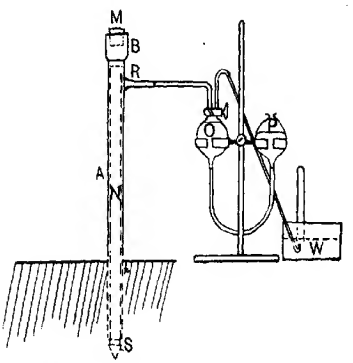


Fig. 15. Apparatus for the collection of soil air.

To obtain a sample of soil air the tube is driven vertically down into the soil to the required depth with a wooden mallet, great care being taken to prevent lateral movements. The inner rod (*N*) is then punched down about $\frac{1}{4}$ in. and a rubber stopper (*M*) inserted in the hole at the top of the tube.

A small bulb (*O*) of approximately 30 c.c. capacity provided with a two way tap is connected to the side tube (*R*) by means of pressure tubing, and also to a small mercury reservoir (*P*); it has a delivery tube attached through which the gas is forced into a mercury trough (*W*) for collection. The flat side of the inner rod allows the gas to pass freely up the tube when the pressure in the bulb is diminished by

lowering the mercury reservoir. The first 20–30 c.c. is rejected and the next 25 c.c. is collected over mercury in thick-walled test tubes, which are then placed in small crucibles and transported in a rack to the laboratory for analysis. To prevent the rack from being blown over by winds it is held firmly in the ground by iron spikes passing through the base pieces. Only one sample is collected at each point. Successive samples vary slightly in composition (Table X) but a fairly large volume of air of tolerably uniform composition can if desired be withdrawn from the same hole.

TABLE X. *Percentage composition of successive* 30 *c.c. samples of soil air drawn from the same hole.*

Hole 1	CO_2	O_2	N_2	Hole 2	CO_2	O_2	N_2
1	0·10	20·74	79·16	1	0·36	20·36	79·28
2	0·10	20·72	79·18	2	0·45	20·46	79·09
3	0·11	20·86	79·03	3	0·39	20·55	79·06
4	0·12	20·63	79·25	4	0·36	20·54	79·10
5	0·14	20·77	79·09	5	0·36	20·57	79·07
6	0·12	20·67	79·21	6	0·36	20·45	79·19
7	0·12	20·71	79·17				
8	0·13	20·80	79·07	Hole 3			
9	0·13	20·68	79·19	1	0·18	20·62	79·20
10	0·13	20·79	79·08	2	0·18	20·74	79·08
11	0·13	20·69	79·18	3	0·15	20·63	79·22
12	0·13	20·76	79·11	4	0·15	20·74	79·61
				5	0·18	20·74	79·08
				6	0·23	20·54	79·23
				Hole 4			
				1	0·26	20·57	79·17
				2	0·25	20·49	79·26
				3	0·25	20·45	79·30
				4	0·23	20·52	79·25
				5	0·23	20·74	79·03
				6	0·21	20·63	79·36

As a rule samples of air from 8–12 holes on each plot are drawn and analysed separately, and the mean value is taken to represent fairly accurately the composition of the soil air. These mean values are given in Table VI and plotted in the various Figures 1 to 6.

Samples were drawn from all the plots on the same day so that the values are strictly comparable. The variation from place to place is fairly large, especially on the plot which has received annually 14 tons of farmyard manure, but on the unmanured plot it is comparatively narrow.

TABLE XI. *Showing variation in percentage composition of soil air taken from different holes on the same plot.*

Hole	Broadbalk (dung)			Hole	Broadbalk (unmanured)		
	CO_2	O_2	N_2		CO_2	O_2	N_2
1	0·39	20·63	78·98	1	0·27	20·69	79·04
2	0·32	20·66	79·02	2	0·19	20·77	79·04
3	0·25	20·76	78·99	3	0·33	20·63	79·04
4	0·37	20·69	78·94	4	0·29	20·64	79·07
5	0·34	20·70	78·96	5	0·38	20·67	78·95
6	0·32	20·69	78·99	6	0·34	20·69	78·97
7	0·41	20·53	79·06	7	0·29	21·09	78·62
8	0·40	20·54	79·06	8	0·26	21·15	78·59
Mean	0·35	20·65	79·00		0·29	20·79	78·92
Probable error of 1 determination	±0·03	±0·06			±0·03	±0·11	
Probable error of mean of all 8	±0·01	±0·02			±0·02	±0·05	

Hoos Field Fallow, Oct. 17, 1913.

Hole	CO_2	O_2	N_2
1	0·19	22·33	77·48
2	0·18	21·27	78·55
3	0·25	21·13	78·62
4	0·34	21·09	78·57
5	0·12	21·12	78·76
6	0·20	21·26	78·54
7	0·12	21·19	78·69
8	0·25	20·99	78·76
Mean	0·21	21·29	78·50
Probable error of 1 determination	±0·04	±0·16	
Probable error of mean of all 8	±0·02	±0·10	

It has frequently been found impossible to obtain a sample of air when the steel rod is driven into the clay subsoil and also when the surface of the ground is frozen. The soil was very wet and the pore space comparatively small, and displacement of the soil air apparently could not take place.

Analysis of soil air. The large type of Haldane's gas apparatus is used. The measuring tube (*A*, Fig. 16) has a capacity of 21 c.c. and is graduated from 15–21 c.c. into 0·01 c.c. The analysis of the gas is carried out under constant pressure; temperature and water vapour pressure are compensated by the bulb shown to the left. The water in the jacket must be thoroughly mixed before readings are taken: this is done by blowing though it air from foot bellows. A laboratory vessel (*B*)

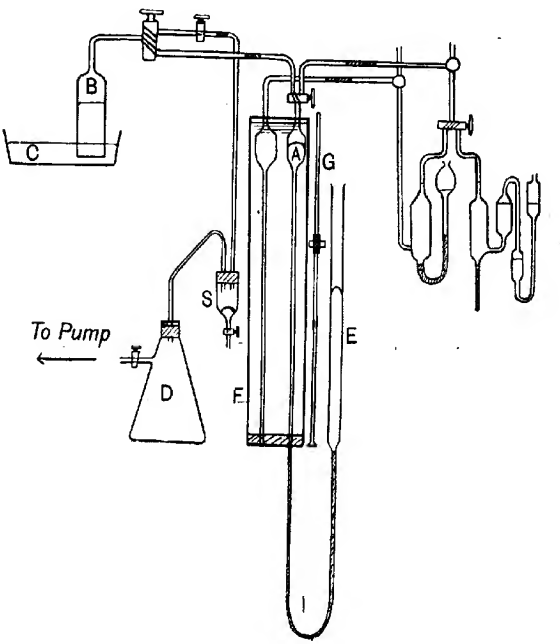


Fig. 16. Apparatus for the analysis of soil air.

in a porcelain mercury trough (*C*) [as used in the well-known Bone and Wheeler gas apparatus] is attached to the measuring tube and filled with mercury by connecting with an evacuated flask (*D*) provided with a mercury trap (*S*). Through this laboratory vessel the gas is readily introduced into the measuring tube. The analysis proceeds in the usual way. Finally the residual gas is forced by means of the levelling tube (*E*) into the laboratory vessel and ejected. A small telescope

sliding on a fixed brass rod (*G*) in front of the water jacket (*F*) which surrounds the measuring tube, and an electric light behind, enable accurate readings to be taken. The laboratory vessel also allows of analyses being made by absorption with small quantities of reagents.

A simple apparatus for teaching purposes. For teaching and demonstration purposes the apparatus in Fig. 17 is very useful. A piece of

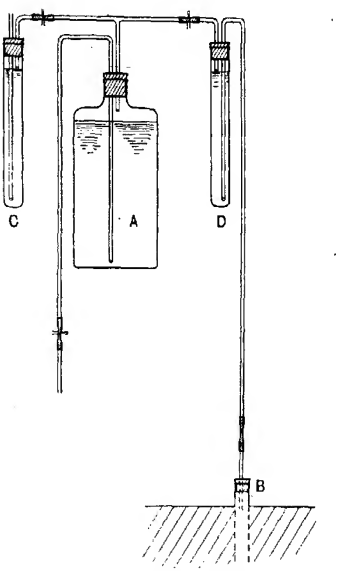


Fig. 17. Apparatus for demonstrating the pressure of CO_2 in soil air.
A, aspirator. *B*, ½″ gas pipe driven into soil.
C, tube of saturated baryta water open to air.
D, „ „ „ „ connected to soil.

half inch gas pipe is driven 6 inches into the soil and connected through a tube of baryta water to a large bottle of water fitted with a syphon tube so that it can act as an aspirator. A second tube of baryta water open to the atmosphere is also attached to the bottle. Set the aspirator going and arrange the clips so that air bubbles pass through both baryta tubes at the same rate. In a short time the one connected with the soil shows turbidity while the other open to the air is still clear.

II. *The soil of the Rothamsted fields.*

All the samples of air dealt with in this paper have been drawn from the Rothamsted fields. The soil is a heavy loam with many stones: it becomes very sticky when wet, but can be got into a good crumbly tilth as it becomes drier. Its mechanical analysis is as follows:

Top 9 inches.

Name of fraction	Diameter of particles	Broadbalk %	Hoos Field %
Fine gravel	3 to 1 mm.	1·9	2·0
Coarse sand	1 to 0·2 mm.	6·2	6·8
Fine sand	0·2 to 0·04 mm.	21·4	19·5
Coarse silt.....	0·04 to 0·01 mm.	32·5	28·9
Fine silt	0·01 to 0·002 mm.	13·8	15·5
Clay.....	less than 0·002 mm.	17·6	18·8

The pore space and space normally occupied by air are:

Soil from	Loss on ignition, %	Specific gravity of dry soil		Volume occupied in natural state by		Volume of water		Volume of air	
		Apparent	True	Solid matter	Air and water spore space	In normal moist state	After period of drought	In normal moist state	After period of drought
Broadbalk unmanured plot	4·3	1·57	2·36	65·9	34·1	23·2	17	10·9	17·1
Broadbalk dunged plot	10	1·46	2·31	61·8	38·2	30·3	20	7·9	18·2

Summary and Conclusions.

1. The free air in the pores of the soil to a depth of 6 inches is very similar in composition to the atmospheric air but it differs in two respects:

(*a*) It contains more CO_2 and correspondingly less oxygen, the average in 100 volumes being 0·25 volume CO_2 and 20·6 of oxygen against 0·03 volume CO_2 and 20·96 oxygen in atmospheric air.

(*b*) It shows greater fluctuations in composition.

Usually the sum of the CO_2 and oxygen is only slightly less than in atmospheric air but at periods when nitrates rapidly increase there is

a perceptible falling off of oxygen, and a still greater one in waterlogged soils.

2. Besides this free air there is another atmosphere dissolved in the water and colloids of the soils. This consists mainly of CO_2 and nitrogen and has practically no oxygen.

3. The fluctuations in composition of the free soil air are mainly due to fluctuations in the rate of biochemical change in the soil, the curves being similar to those showing the amount of nitrate and the bacterial counts as far as they were taken. The rate of biochemical activity attains a maximum value in late spring and again in autumn, and minimum values in summer and winter. In autumn the bacteria increase first, then the CO_2 rises, and finally the nitrate increases.

From November to May the curves closely follow those for the soil temperature which thus appears to be the dominating factor; from May to November they follow the rainfall and to a less extent the soil moisture curves. The distinct difference between rainfall and soil moisture indicates that rainfall does something more than add water to the soil. It is shown that the dissolved oxygen brought in is probably a factor of considerable importance in renewing the dissolved soil atmosphere and facilitating biochemical change.

4. Grass land usually contains more CO_2 and less oxygen than arable land but we cannot attribute the difference to the crop owing to the large differences in soil composition and conditions. It is difficult to ascertain the precise effect of a crop, but as the soil differences are eliminated so the differences in composition of the soil air become less and less. No evidence could be obtained that the growing crop markedly increases the amount of CO_2 in the soil air, and if it gives rise to any great evolution of CO_2 in the soil it apparently exercises a corresponding depressing effect in the activities of soil bacteria. This result agrees with one obtained earlier in reference to the nitrates in the soil.

5. Such weather conditions as barometric pressure, wind velocity, variations in temperature from the mean, small rainfall, etc. seem to have but little effect on the soil atmosphere.

TABLE VI (*a*). *Composition of soil air (mean percentages by volume) and meteorological data.*

ivated land oadbalk lderness		Arable land												Meteorological data				
		Broadbalk wheat						Hoos wheat and fallow										
		Dunged			Unmanured			(1)			(2)			Rain-fall for 7 previous days	Mean temps for previous day		Baro. mms.	Character of previous day
O_2	N_2	CO_2	O_2	N_2	CO_2	O_2	N_2	CO_2	O_2	N_2	CO_2	O_2	N_2		air	soil 6″		
20·35	79·10	—	—	—	—	—	—	—	—	—	—	—	—	0·38″	4·4° C.	—	748·5	Showery and dull till midday; then fine and frosty at night.
20·40	79·32	—	—	—	—	—	—	—	—	—	—	—	—	1·22	4·4	—	748·7	Dull; snow melted by 9.0 a.m.; much milder; S. wind.
20·45	79·15	—	—	—	—	—	—	—	—	—	—	—	—	0·14	4·4	—	752·2	Fair morning, sharp rains in afternoon and early evening; S.W. winds.
20·17	79·43	0·55	18·97	80·48	0·13	19·86	80·01	—	—	—	—	—	—	0·60	8·3	—	766·2	Fair, bright in afternoon; S.W. winds.
20·16	79·51	0·22	20·24	79·54	0·12	20·72	79·16	—	—	—	—	—	—	0·30	4·8	—	748·0	Fair till midday; little rain at noon; fair; S.E. light winds.
—	—	0·31	20·44	79·25	0·10	20·77	79·13	—	—	—	—	—	—	0·33	10	—	753·2	Fair day, some rain with gusty winds at night.
19·87	79·63	0·46	20·18	79·36	0·21	20·53	79·26	—	—	—	—	—	—	0·22	3·9	—	754·2	Fine, light S. winds.
20·19	79·26	0·65	19·70	79·65	0·22	20·64	79·14	—	—	—	—	—	—	0·68	1·7	—	754·7	Fair, cold N. winds, bright sunshine at times.
20·34	79·09	0·89	19·93	79·18	0·22	20·71	79·07	—	—	—	—	—	—	0·56	11·1	—	749·2	Fine, warm, light E. winds.
19·50	79·46	2·27	17·61	80·12	0·55	20·19	79·26	—	—	—	—	—	—	1·29	10	—	750	Fair generally; showery evening; fine frosty air at night; S. to S.W. winds.
18·82	79·45	1·45	19·42	79·13	0·35	20·53	79·12	—	—	—	—	—	—	0·56	10·5	11·1	756·2	Fair morning, showery from noon onwards till evening.
20·69	79·59	0·42	20·56	79·02	0·50	20·77	78·73	0·51	20·39	79·10	0·10	20·82	79·08	0·35	13·9	17·4	755·5	Fine; S. winds.
20·97	78·67	0·35	20·66	79·01	0·29	20·79	78·92	0·36	20·64	79·00	0·27	20·66	79·07	0·42	15	15·5	752·2	Dull; mild: thunder in S. from 1.30 to 3 p.m.; some showers; fine later.
20·62	79·01	0·24	20·70	79·06	0·22	20·73	79·05	0·20	20·70	79·10	0·09	20·84	79·07	0·24	19·4	18·1	750	Fine hot morning; light N.E. wind; fine and very hot.
20·57	78·97	0·17	20·79	79·04	0·11	20·83	79·06	0·26	20·80	78·94	0·05	21·01	78·94	0·52	14·4	13·3	757·2	Fine and bright; warmer N.W. wind very light; little fog at night.
20·47	79·00	0·18	20·81	79·01	0·16	20·82	79·02	0·30	20·47	79·23	0·07	20·85	79·08	0·41	11·1	12·9	745	Changeable, sprinkles of rain in afternoon; S. breezes.
20·50	78·80	0·34	20·43	79·23	0·16	20·72	79·12	0·38	20·61	79·01	0·21	21·30	78·49	0·47	12·2	12·0	759	Fair, light W. winds; good deal of cloud in afternoon and evening; misty at night.
20·62	78·80	0·54	20·72	78·74	0·35	20·56	79·09	0·33	21·06	78·61	0·11	20·90	78·99	0·21	5·0	6·5	744·7	Fine; S.W. light airs, some rain at night.

20·17	79·19	0·35	20·47	79·18	0·29	20·27	79·44	0·32	20·57	79·11	0·10	20·76	79·14	0·29	7·2
20·19	79·28	0·34	20·45	79·21	0·25	20·35	79·40	0·17	20·44	79·39	0·04	20·68	79·28	0·13	2·8
20·55	79·13	0·32	20·51	79·17	0·14	20·62	79·24	0·10	20·92	78·98	0·16	20·88	78·96	0·09	0·5
—	—	0·34	20·52	79·14	—	—	—	—	—	—	—	—	—	0·19	7·2
20·42	79·26	0·24	20·54	79·22	0·26	20·33	79·41	0·04	20·85	79·11	0·15	20·77	79·08	1·27	7·8
—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·07	6·1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·72	3·3
19·56	79·95	0·30	20·16	79·54	0·29	20·28	79·43	0·03	20·69	79·28	0·03	20·75	79·22	2·12	3·3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·40	8·9
19·65	79·87	0·76	19·31	79·93	0·34	19·85	79·81	0·02	20·78	79·20	0·10	20·58	79·32	0·68	8·9
20·44	79·18	0·65	20·08	79·27	0·81	19·98	79·21	—	—	—	—	—	—	0·04	10
20·29	79·26	0·44	20·22	79·34	0·28	20·45	79·27	0·05	20·73	79·22	0·18	20·48	79·34	0·71	6·1
—	—	0·61	—	—	0·40	—	—	0·10	—	—	0·24	—	—	0·16	12·8
—	—	0·32	—	—	0·42	—	—	0·07	—	—	0·29	—	—	0·26	10
—	—	0·40	—	—	0·36	—	—	0·08	—	—	0·28	—	—	0·84	11·7
—	—	0·48	—	—	0·37	—	—	0·07	—	—	0·41	—	—	1·49	13·3
20·21	79·28	0·43	20·39	79·18	0·50	19·80	79·70	0·10	20·62	79·28	0·43	19·89	79·68	1·38	17·2
20·35	79·18	0·48	20·36	79·16	0·47	20·29	79·24	0·08	20·54	79·38	0·42	20·15	79·43	0·88	14·4
—	—	0·30	—	—	0·35	—	—	0·09	—	—	0·24	—	—	0·25	15·0
20·50	79·18	0·24	20·61	79·15	0·24	20·54	79·22	0·04	20·72	79·24	0·13	20·70	79·17	0·32	15·0
20·52	79·20	0·31	20·53	79·16	0·22	20·60	79·18	Up to Oct. 6, 1913 (1) is Hoos Wheat and (2) Hoos Fallow. After Oct. 6, 1913 (1) is Hoos Fallow and (2) Hoos Wheat. Hoos Wheat of 1913 ploughed up between Dec. 22, 1913 and Jan. 20, 1914 for the 1914						0·33	10

6·9	758	Dull, mild, damp at times; S.E.
4·5	763·5	Dull; slightly misty; E. light breezes slight frost at night.
2·4	753·5	Dull, cheerless day, cold N.E. winds; snow midday; sleet in evening.
4·6	751·7	Fair, mild, light S. breezes, sprinkle of rain about 1 p.m.
6·5	743·5	Dull with rain all day; heavy showers at night; S. to S.E.
5·8	753·2	Fair day, cool W. winds; shower about 7.30 p.m.; then fair again.
4·7	751·5	Fine morning; cold; snow; fine later; sharp white frost.
4·7	744·2	Fair or fine during day; rain at night; W winds.
7·4	759	Showery; W. winds.
10·1	741·2	Fair during the day; heavy showers from 7 p.m. onwards; W. wind.
14·1	756	Fine and bright; E. airs cool at night.
9·1	752·2	Dull and cold; drizzling rain at times; W breezes light.
14	760·7	Fine; warmer; S.W.
14·6	760·2	Fair generally, cold, some showers in after noon; N.W. to N.
15·8	749	Fair morning and mild; dull later; colder continuous rain after 7 p.m.; N.W.
16·3	752·2	Fine, fresh S. to S.E. breezes till 3 p.m. then heavy rain till 8 p.m. ; N. to N.W
16·1	753·2	Dull, misty at early morn; bright periods in afternoon; N.W. light breezes.
18	752·2	Showery at times, S. to S.W. light breezes sharp shower about 7 p.m.
17·4	743·5	Cool; good deal of cloud; one or two showers between 2 and 4 p.m.
16·2	750·5	Showery during morning, fine later, cool at night; S. wind.
12·2	760·7	Fair generally; slight showers about noon and 6 p.m.; cold N.W. winds.

TABLE VI (*b*). *Moisture content, nitrate content, and bacterial numbers in soils on dates of sampling.*

Date	Soil moisture per cent.					N as nitrate, parts per million					Bacterial numbers. Millions per gram				
	Broadbalk			Hoos		Broadbalk			Hoos		Broadbalk			Hoos	
	Wilderness	Dunged	Unmanured	Wheat	Fallow	Wilderness	Dunged	Unmanured	Wheat	Fallow	Wilderness	Dunged	Unmanured	Wheat	Fallow
June 3, 1913	12	11	7	11	11	6	9	3	3	7	—	—	19	9	4
July 11 „	15	11	8	9	8	7	7	6	4	18	—	21	9	6	11
Aug. 29 „	12	10	5	6	4	4	14	7	—	15	19	14	7	6	8
Sept. 22 „	17	16	13	13	12	6	17	6	3	15	26	30	4	11	12
Oct. 6 „	21	18	15	15	14	3	18	8	3	20	9	29	17	9	11
Oct. 17 „	19	20	15	16	15	5	17	7	14	3	17	24	15	7	15
Nov. 10 „	20	22	17	16	16	4	15	6	8	4	20	30	18	13	8
Nov. 22 „	—	19	16	—	—	—	13	3	—	—	—	—	—	—	—
Dec. 9 „	21	21	17	16	15	15	22	16	16	12	22	35	12	9	8
Dec. 22 „	18	21	16	16	15	9	29	20	14	11	12	30	22	9	9
Jan. 8, 1914	18	23	17	17	17	20	29	17	20	18	13	26	8	7	6
Jan. 20 „	20	27	16	16	16	18	25	18	17	16	14	25	10	14	9
Jan. 30 „	18	21	17	17	17	7	14	9	9	13	13	—	11	11	11
Feb. 12 „	20	24	16	15	17	4	19	3	5	4	—	—	—	—	—
Mar. 2 „	20	21	16	16	16	—	—	—	—	—	—	—	—	—	—
Mar. 11 „	21	22	16	6	7	7	14	7	4	7	—	—	—	—	—
Mar. 31 „	21	22	16	16	16	8	15	9	12	10	—	—	—	—	—
April 30 „	—	16	11	—	—	—	27	24	—	—	—	—	—	—	—
May 11 „	19	18	13	12	13	—	—	—	—	—	12	9	8	8	8
May 18 „	—	12	11	—	—	—	12	12	—	—	—	—	—	—	—
May 25 „	15	13	11	9	11	10	11	10	11	17	12	24	11	7	6
June 10 „	18	18	13	13	12	11	19	12	12	15	—	—	—	—	—
June 12 „	18	19	15	16	15	—	—	—	—	—	—	24	16	14	20
June 26 „	—	13	11	—	—	—	—	—	—	—	—	—	—	—	—
July 7 „	16	17	12	13	13	6	15	15	12	17	29	31	16	15	10
July 21 „	—	14	9	—	—	—	11	9	—	—	—	—	—	—	—
July 27 „	9	9	5	6	7	7	6	5	7	16	—	10	7	6	8
Aug. 13 „	—	14	11	—	—	—	6	7	—	—	—	—	—	—	—
Sept. 12 „	15	15	11	8	10	6	12	9	14	19	—	13	8	6	4
Sept. 21 „	16	16	11	—	—	4	3	8	—	—	—	16	7	—	—

Between Oct. 6 and 17 Hoos Wheat of 1913 becomes Hoos Fallow 1914.

STUDIES ON SOIL PROTOZOA.

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I. Introduction.

The work discussed in this paper is a continuation of that described in a previous communication to the *Centralblatt für Bakteriologie*[1]. Unfortunately it has been found impossible to bring all the problems taken up to a satisfactory conclusion in the time available. And as, owing to unforeseen circumstances it is necessary, for the present at least, to leave this subject, the results so far as they have been arrived at are brought together in this publication and the lines on which it had been intended to work are indicated. It is hoped, however, that an opportunity of continuing this work on the soil Protozoa may present itself at some future date.

The points which will be dealt with here are:

The dilution method and its application to the enumeration of protozoa in soils.

The effect of protozoa on the numbers of bacteria in ammonifying solutions and on ammonification in solution tests.

The effect of inoculations of protozoa on the bacterial content of partially-sterilised soils.

II. The Dilution Method.

The dilution method has already been applied to the enumeration of protozoa in soils by Rahn[2]. He used as media peptone and sugar solutions incubated for 7–14 days. The dilutions were made in the usual way and at the end of the incubation period the cultures were

[1] *Centralbl. f. Bakt.* Abt. II. Bd. **39**, pp. 596–610.

[2] *Ibid.* Bd. **36**, p. 419.

submitted to microscopic examination. As a result of his work he found that drying the soil caused a reduction in the numbers of protozoa and that this reduction was first noticeable in the case of the amoebae. Killer[1] also used the dilution method, with a number of the solutions employed for cultivation of soil bacteria as media.

The method employed in these experiments is in principle the same as that generally applied in bacteriological work. Four parallels in each dilution are used. The medium is soil extract (prepared as described in Löhnis' *Praktikum*, p. 118, but undiluted) + ·1 % K_2HPO_4 in 1 c.c. quantities in small test-tubes. To each tube 1 c.c. of the dilution-water is added, so that the medium, so far as the protozoa are concerned, is ordinary soil extract + ·05 % K_2HPO_4. But, if the dilutions are put up simply as above described, it has been found that the multiplication of the protozoa after excystation is rather slow and the microscopic work as a consequence is very tedious. It has been observed that inoculation of the soil extract with a protozoa-free culture of bacteria, prepared from a bloodmeal culture as described in the previous paper (p. 604), hastens the multiplication of the protozoa. The microscopic work is thus considerably facilitated. The procedure is to inoculate the soil extract with the protozoa-free culture and incubate for two days before inoculation from the dilutions.

Subsequent work on the effects of moisture, etc. on the protozoal content of soils has shown that the dilutions 100, 1000, 10,000, 100,000, etc., are not close enough to bring out differences due to the treatment of the soils. It has, therefore, been found necessary to employ closer dilutions. Those used are, for example, 100, 300, 500, 750, 1000, 3000, 5000, etc.

When now the method is applied to the enumeration of protozoa in soils, it is found that the results are rather irregular. Up to a certain dilution all four parallels in each case give positive results. Then in the next three or four dilutions 1–3 of the parallels in each are positive, the remainder negative. Table 1 shows a typical case. The figures given in the columns indicate the number of parallels showing positive results.

It will be observed that after five days' incubation the development is regular up to and including 5000 with single positive results in each of the next four dilutions. On incubation for a further period of 25 days the regular development stage is pushed forward to the 10,000 dilution. But beyond this irregularities still remain. From the point

[1] *Centralbl. f. Bakt.* Abt. II. Bd. 37, p. 521.

of view of regularity, therefore, the 30 days' is no better than the five days' period, although it gives a slightly higher result. But 30 days is as long an incubation period as could be conveniently adopted and consequently it was not considered advisable to investigate the effects of a longer period. And, as five days' incubation gives as satisfactory results as 30 days, the former has been adopted in subsequent work. The question of the higher results obtained after 30 days need not be considered in view of the much greater convenience of the shorter period. In any case the dilution method, here as with bacteriological work, gives only relative, not absolute results. The whole of the protozoa in soils do not develop in soil extract.

TABLE 1.

Incubation period (22° C.)	Dilutions					
	5000	7500	10,000	30,000	50,000	75,000
5 days ..	4	1	1	1	1	0
12 „ ..	4	3	3	2	2	0
30 „ ..	4	4	4	3	2	2

With a view to obtaining more regularity in the results some slight modifications of the method were tried. In the dilution method, as used in bacteriological work, the addition of small quantities of sterile soil to the medium is found to have a beneficial effect on the growth of the soil bacteria. It was thought that the same might apply to the protozoa but this has not proved to be the case. Indeed, the use of sterile soil results in a much slower excystation of the protozoa and no improvement as regards regularity. The retardation of excystation is probably due to the extraordinarily beneficial effect which the soil has on the growth of the protozoa-free culture before the inoculation with protozoa. It has frequently been observed that the development of protozoa in a medium containing exceptionally large numbers of bacteria is considerably hindered. In a further experiment the inoculation with protozoa-free culture was omitted, only sterile soil being added. The results obtained were very low and quite as irregular as in the previous case.

The effect of the reaction of the medium was next tested. But soil extract + chalk as well as soil extract + ·01 % hydrochloric acid and soil extract + ·01 % caustic potash effected no improvement with

regard to the regularity of the results. The ordinary soil extract + ·1 % K_2HPO_4 + protozoa-free culture was therefore used in all subsequent work and the last dilution in which all four parallels gave a positive result after five days' incubation at 22° C. was adopted (quite arbitrarily, of course) as the protozoal content of the material examined. In all cases the results are given as numbers per gram of soil.

With regard to the cause of the irregularity in the development in the dilutions, it is most probably to be explained on the supposition that the protozoa adhere very readily to the soil particles. It is exceedingly likely that the amoebae in particular may be carried over from dilution to dilution in this way.

In the last paper, 58° C. was suggested as a temperature which would kill off all active soil protozoa capable of development on soil extract and so allow of a *distinction* being drawn *between active and encysted forms*. This temperature has been adopted in combination with the dilution method already described. Two sets of dilutions are generally made, the first with the untreated soil, the second with the soil after heating to 58° C. The heating is generally carried out in the 100 dilution.

TABLE 2.

	Dilutions				
Sample A	750	1000	3000	5000	7500
Total Nos.	4	4	2	2	2
After heating to 58° ..	4	3	1	0	0
Sample B	100	300	500	750	1000
Total Nos.	4	4	4	4	3
After heating to 58° ..	4	4	3	2	2

As a result of further work it appears probable that a temperature of 58° C. kills a number of the encysted protozoa in addition to the active forms. Thus, for example, it has been found that pure cultures of certain flagellate and ciliate cysts do not excyst after being heated to 58° C. and subsequently brought into fresh media. The results of some experiments on the effect of drying on the protozoa may also be cited in this connection. Two samples of soil were allowed to dry at

22° C., A for nine days, B for 16 days. Protozoa counts were then made as above described. Table 2 shows the results.

In these cases as a result of drying one would naturally expect at least a certain number of the protozoa to encyst and the total numbers to be equal to those obtained after heating to 58° C. provided the latter treatment had no injurious action on the cysts. But in both cases the heating appears to have destroyed a number of the encysted organisms. In the first case the distinction is small but in the second it is considerable.

In order to obtain further evidence on this point the effect of treatment with caustic potash on cysts and active forms was examined. As a preliminary experiment soil extract cultures of protozoa showing numerous active and encysted forms were treated for varying lengths of time with equal quantities of a ·5 % caustic potash solution, so that the concentration of the alkali in the cultures was ·25 %. At the end of the period of treatment a drop of phenolphthalein solution was added to each culture and the potash neutralised with dilute lactic acid. The cultures were allowed to settle for half-an-hour and then examined microscopically. After five days' incubation at 22° C. they were examined once more. The results are given in Table 3. + indicates the presence of active organisms.

TABLE 3.

Interval since Neutralisation	Controls (with addition of phenolphthalein)		Potash allowed to act for:					
			1 minute		1 hour		4 hours	
	A	B	A	B	A	B	A	B
Half hour	+	+	–	–	–	–	–	–
Five days	+	+	+	–	+	+	+	+

The treatment with potash killed all active protozoa but left the encysted uninjured and the latter were able to excyst within five days.

The treatment with ·25 % potash for one hour was applied in the dilution method. Dilutions were made from a sample of soil, in the one case after the soil had been heated to 58° C. in the 100 dilution, in the other after it had been treated with ·25 % potash for one hour, also in the 100 dilution. Table 4 shows the results.

Here it will be observed that the heat has had a more drastic action on the cysts than has the potash. It appeared possible that this

distinction might be due to the adsorption of some of the potash by the soil. It was found, however, by titration of the remaining alkali with acid that, under the conditions of the above described experiments, only 10–15 % of the potash was put out of action,—a quantity quite insufficient to account for the difference in the results obtained.

TABLE 4.

Soil	Dilutions				
	500	750	1000	3000	5000
(1) Heated to 58° C. ..	4	1	2	1	0
(2) Treated with ·25 % KOH for one hour ..	4	4	3	2	3

From these experiments, therefore, it seems highly probable that heating to 58° C. kills a considerable number of the encysted protozoa. But it has been shown that heating to 58° C. is absolutely necessary if one wishes to make sure of killing off all active forms (particularly ciliates). From what has been said it is evident that it is impossible to fix upon a temperature which will destroy all active protozoa in soils and leave the cysts perfectly uninjured. This was only to be expected. In the case of the bacteria the power of resistance to heat of the active forms varies enormously and sometimes even surpasses that of the spores of less resistant species. The same remark would appear to apply to the protozoa. Further, it must be remembered that during a period of excystation or encystation of a particular species it is quite impossible to draw a hard and fast distinction between cyst and active form. And it is obvious that the various transition forms encountered in such cultures must have very varied powers of resistance to heat. Any temperature selected for the purpose of distinguishing active protozoa from cysts must therefore be of an arbitrary nature. And as it is better to select a temperature which will kill all active forms even if it does injure some of the cysts, rather than one which will leave the cysts unharmed and also probably some of the active forms alive, the continued use of 58° C. seems to be justified. This view is supported by the results of experiments which will presently be discussed. It has been found that the numbers obtained by the dilution method after heating to 58° C. (referred to, later, as "Cysts") show variations corresponding with variations in the treatment of the material. The method,

therefore, yields useful results which, after all, is the best justification it can have.

The results of the experiments on the effect of heat quoted above probably rather exaggerate its injurious action. In this connection three points must be kept in mind:

(1) It must be remembered that in those cases in which the cysts failed to excyst after heating to 58° C. pure cultures were dealt with. In the results of experiments on the effect of heat on cysts, described in the previous paper, the thermal death point of the most resistant cysts found in soil is given as 72° C. This does not, however, exclude the possibility of the presence of forms with less resistant cysts.

(2) In the experiments on the effect of drying, the desiccation itself may have had an injurious action on the cysts and as a consequence may have rendered them a more easy prey to the injurious influence of the heat.

(3) In the potash experiment, protozoa which had been cultivated on an artificial medium (soil extract) and thus probably rendered less resistant, are dealt with.

III. The Occurrence and Activity of Protozoa in Soils as indicated by the Dilution Method.

The relative occurrence of the flagellates and amoebae in soil is indicated in Table 5.

TABLE 5.

Dilutions.....	3000	5000	7500	10,000
Parallels	A B C D	A B C D	A B C D	A B C D
F=flagellates A=amoebae	F F FA F	FA FA F F	FA F F F	F FA F F

Dilutions.....	30,000	50,000	75,000	100,000
Parallels	A B C D	A B C D	A B C D	A B C D
F=flagellates A=amoebae	F F — F	F — — —	— — — F	F — — —

The flagellates are seen to occupy first place. The amoebae are rather fewer in number but this may be due to the fact that they are

rather sensitive to the presence of members of the other groups and are probably to some extent suppressed by them in the cultures. The ciliates are always present in much smaller numbers. They are rarely seen in dilutions exceeding 100. But the appearance of the various groups in particular dilutions cannot be considered as giving any very sure indication of the relative occurrence of protozoa in soils.

To the question as to whether the protozoa lead an active life in soil, it has been shown that the action of heat combined with the dilution method does not give a definite answer. That question, however, is answered in the affirmative by the results of experiments which will now be discussed.

(*a*) *The Effect of Temperature.*

(i) *On the number of protozoa in soils.* For these experiments some garden soil was passed through a 2 mm. sieve and placed in an ordinary porous flowerpot. The moisture content was determined and adjusted to 70 % of the water-holding capacity of the soil. It was kept at this degree of moistness by watering with boiled water every day during

TABLE 6.

Total Numbers	Dilutions				
	7500	10,000	30,000	50,000	75,000
In original soil	4	4	2	—	—
After 9 days at 5–7° C. ..	4	4	3	1	1
After further 7 days at 22° C.	4	4	4	3	2
After further 7 days at 30° C.	4	4	3	2	0
Cysts	500	750	1000	3000	5000
In original soil	4	4	3	2	—
After 9 days at 5–7° C. ..	4	4	4	1	2
After further 7 days at 22° C.	4	4	4	2	2
After further 7 days at 30° C.	4	4	3	4	3

the course of the experiment. The total numbers of protozoa and cysts growing on soil extract were determined by the method described above, immediately after the first adjustment to 70 % W.H.C. The pot was kept in succession for nine days at 5–7° C., for seven days

at 22° C. and for seven days at 30° C., determinations of total numbers of protozoa and cysts being made before each change of temperature. Table 6 shows the results.

It will be noted that after nine days at 5–7° C. practically no change from the original numbers is observed. This is as was to be expected, for the temperature was about the same as that to which the soil had been exposed in the garden and the only change in the conditions was that the soil in the flowerpot had received about 3 % more water than was present in the plot from which it was taken. But after a period of seven days at 22° C. quite a considerable increase in the total number has taken place while the cysts have remained practically stationary. Exposure to a temperature of 30° C. for seven days has caused a fall in the total numbers but a distinct rise in the number of cysts. The fall in the total numbers is readily explained when one bears in mind that certain of the soil protozoa in active form are killed by a temperature of 25° C. Doflein[1] refers to the work of Grosse-Allermann who showed that *Amoeba terricola* (Greef) is killed after a few hours at 25° C. But apart from this 30° C. is evidently too high a temperature to allow of the activity of quite a number of the protozoa in soils as is shown by the increase in the number of cysts. As the result of these experiments, therefore, a temperature in the neighbourhood of 22° C. seems to be the most suitable for the activity of the majority of the soil protozoa.

But although 22° C. is the optimum for the majority of the protozoa in soils, it does not exclude the possibility of the presence of other organisms adapted to higher temperatures. In order to try to throw some light on this point a further experiment was undertaken. The protozoal content of a sample of soil which had been saturated with water and kept for eight days at 22° C. was determined. The soil was then placed in the 30° C. incubator for 38 days, during which time it was kept saturated with water. Determinations of the numbers of protozoa in the soil after eight and 38 days respectively were made. For all three determinations quantities of the soil corresponding to the same dry weight were employed so that the figures in Table 7 are comparable.

A fall in the total numbers of protozoa is observed after eight days at 30° C. as was to be expected from the results given above. But later the organisms which are adapted to the higher temperature show a marked increase in numbers. It is evident, therefore, that soil

[1] *Lehrb. d. Protozoenkunde*, p. 319.

contains protozoa adapted to a temperature of about 30° C. and that these become active when the conditions are favourable for their growth.

TABLE 7.

Total Numbers	Dilutions				
	10,000	30,000	50,000	75,000	100,000
After 8 days at 22° C. ..	4	4	3	2	1
After 8 days at 30° C. ..	4	1	2	2	1
After 38 days at 30° C. ..	4	4	4	2	—
Cysts	300	500	750	1000	3000
After 8 days at 22° C. ..	—	—	—	3	2
After 8 days at 30° C. ..	—	4	3	1	2
After 38 days at 30° C. ..	1	2	2	2	0

(ii) *On the kind of protozoa in soils.* Observations on cultures (chiefly bloodmeal solutions + K_2HPO_4) of soil protozoa kept at various temperatures have yielded some interesting results. In such cases at temperatures below 8° C. flagellates only have been observed. These appear to multiply much more rapidly at the low temperature than they do at 22° C., for example, and they continue for a much longer period in the active form. In cultures kept at 22° C. flagellates, ciliates and amoebae may all be present. At 30° C. on the other hand the fauna of culture solutions consists practically entirely of ciliates. A few flagellates are sometimes observed at first. At 38° C. few protozoa develop. Only amoebae have been observed. These points are of importance from the point of view of securing pure cultures of the respective groups.

As to the effect of temperature on the kind of organisms leading an active life in soil, little definite information has been obtained. Ciliates (in addition to flagellates) have been observed directly under the microscope in droplets taken from the surface of a saturated soil kept at 30° C. The forms seen belonged to the genus *Balantiophorus*. Such organisms may, therefore, be of importance in sewage and water-logged soils during hot summer weather in temperate climates and also in the rice-fields of tropical countries.

(b) *Effect of Moisture.*

(i) *On the number of protozoa in soils.* In the following experiments the temperature was kept constant at 22° C.

Experiment I. The water content of a sample of garden soil was adjusted to 70 % of its water-holding capacity and a determination of the number of protozoa present was made by means of the dilution method. The sample was divided into three portions. In the first case 10 grams of the soil was placed in a petri dish. The lid was kept raised so as to allow of evaporation of moisture but prevent contamination from the air. The second portion consisted of 30 grams of soil, also in a petri dish. This sample was saturated with water and the lid allowed to remain in position to prevent evaporation as much as possible. The third portion consisted of the remainder of the sample in a flowerpot covered with cotton-wool to minimise evaporation but allowing free access of air. The first portion was allowed to dry for nine days, the second was kept saturated for eight days, while the third was kept at 70 % W.H.C. for seven days. At the end of these periods a determination of protozoa was made for each portion. In the case of the dry and saturated samples quantities corresponding to one gram of the 70 % sample were taken for the dilutions. The results are shown in Table 8.

TABLE 8.

Total Numbers in	Dilutions						
	1000	3000	5000	7500	10,000	30,000	50,000
Original sample ..	4	4	4	4	4	2	—
Dried sample ..	4	2	2	2	0	1	—
70 % W.H.C. sample	4	4	4	4	4	4	3
Saturated sample	4	4	4	4	4	4	3
Cysts in	300	500	750	1000	3000	5000	7500
Original sample ..	4	4	4	3	2	—	—
Dried sample ..	—	—	4	3	1	0	0
70 % W.H.C. sample	4	4	4	4	2	2	1
Saturated sample	—	—	—	3	2	0	1

The effect of drying is seen in the reduction of the total numbers. The 70 % and saturated samples have given the same increase. The

cysts show practically no change as a result of the variations in the treatment.

Experiment II. In this case the original sample was divided into two portions. One was allowed to dry for nine days: the other was kept saturated for seven days. Bacterial counts were also made on the samples, agar at 22° C. being used as medium. Otherwise the procedure was the same as in Experiment I. Table 9 shows the results.

TABLE 9.

Total Numbers in	Dilutions						Bacteria (millions per gram)
	750	1000	3000	5000	7500	10,000	
Original sample ..	—	—	—	4	2	2	13·95
Dried sample ..	4	3	2	2	—	—	6·90
Saturated sample	—	—	4	4	3	3	5·20
Cysts in	100	300	500	750	1000	3000	
Original sample ..	—	—	4	1	2	1	—
Dried sample ..	—	3	2	3	1	0	—
Saturated sample	—	1	1	2	2	0	—

Drying has again resulted in a reduction in the number of protozoa while the saturation of the soil with water has produced a slight increase in total numbers and a very decided decrease in the number of cysts. The bacterial content has in both cases fallen considerably and it is noteworthy that from the saturated soil more bacteria have disappeared than from that which has been exposed to drying. But the conditions in the saturated soil cannot be regarded as very unfavourable for bacterial growth, for the layer of soil and water is quite a thin one (about $\frac{1}{4}$ inch).

Experiment III. The plan in this case was similar to that adopted in Experiment I, but bacterial counts on agar at 22° were added. The dried soil was kept for 16 days: the 70 % sample for 15 days and the saturated sample for 14 days. Table 10 contains the results.

Here the drying has caused no decrease in the total numbers of protozoa. The latter appear all to have been able to encyst before the soil became too dry for active life. This view is supported by the

TABLE 10.

Total Numbers in	Dilutions						Bacteria (millions per gram)
	750	1000	3000	5000	7500	10,000	
Original sample ..	4	3	2	1	1	0	8·1
Dried sample ..	4	3	—	—	—	—	3·1
70 % W.H.C. sample	4	4	4	3	1	2	6·4
Saturated sample	4	4	4	4	4	4	6·7
Cysts in	50	100	300	500	750	1000	
Original sample ..	0	1	0	0	0	0	—
Dried sample ..	4	4	4	3	2	2	—
70 % W.H.C. sample	3	2	0	0	0	0	—
Saturated sample	3	1	1	0	0	0	—

fact that the cysts have shown a marked increase in the dried sample. Very decided increases in total numbers are observed in the 70 % and saturated samples, especially the latter. The fall in the bacterial content of the 70 % and saturated samples is not so marked in this instance as it was in the case of the saturated sample in Experiment II. This is probably due to the fact that the protozoal activity had reached its maximum before the counts were made as is indicated by the increase in the number of cysts. The same cause has probably resulted in an obliteration of any difference, which might have been expected, in the bacterial contents of the 70 % and saturated samples as a result of the difference in the protozoal content of the latter.

Experiment IV. The 70 % sample, after use in Experiment III, was employed as the starting point in this experiment. The samples to be dried and saturated respectively were taken from it and the remainder was kept for a period of 12 days at 70 % W.H.C. The dried sample was kept for 14 days, the saturated sample for 12 days, and bacterial and protozoal counts were made for all three samples as in the last experiment. The results obtained are given in Table II

Drying has, in this instance, lowered the numbers of protozoa present, while the cysts again remain considerably behind the total numbers. The 70 % sample is, at the end of the experiment, in practically the same condition as it was at the beginning. It is obvious, therefore, that the protozoa in the sample had reached the maximum of their activity during the course of the preceding experiment. Thus,

these results confirm those of Experiment III. The saturated sample in Experiment IV has again shown a great increase in the numbers of protozoa.

TABLE 11.

Total Numbers in	Dilutions									Bacteria
	500	750	1000	3000	5000	7500	10,000	30,000	50,000	
Original sample	—	4	4	4	3	1	2	—	—	6·4
Dried sample	4	3	1	2	—	—	—	—	—	3·8
70 % w.h.c. sample ..	—	—	4	3	3	1	2	3	—	8·0
Saturat'd s'mple	—	—	—	4	4	4	4	4	4	7·2
Cysts in	50	100	300	500	750	1000	3000			
Original sample	3	2	0	0	0	0	0	—	—	—
Dried sample	—	3	1	1	0	0	0	—	—	—
70 % w.h.c. sample ..	2	3	1	0	—	—	—	—	—	—
Saturat'd s'mple	2	3	1	0	—	—	—	—	—	—

The increase in the bacterial content of the saturated sample as compared with the bacterial content of the 70 % sample at the beginning of the experiment is again probably due to the protozoal activity having passed its maximum. The increase in the bacterial numbers in the 70 % sample during the course of the experiment was only to be expected from what has already been deduced.

(ii) *On the kind of protozoa in soils.* In the cultures made from the dilutions, considerable variations are to be observed in the kind of organisms obtained from saturated, 70 % w.h.c. and dry soils. In cultures from saturated soils practically only flagellates are found. The 70 % and dried samples on the other hand yield amoebae in addition to flagellates. Ciliates are seldom seen in any of the cultures. It seems highly probable, therefore, that the flagellates may require a rather moist medium for the unfolding of their activities. The amoebae appear to prefer a somewhat drier soil. But it is possible that they may also lead an active life in saturated soils but may be suppressed in the cultures by the flagellates which are present in large numbers in such soils.

To summarise the results of these experiments on the effects of temperature and moisture on the soil protozoa:—*It has been shown that some, at least, of the protozoa in soils lead an active life and are capable of multiplying to quite a considerable extent when the conditions become favourable. It is also very probable that those protozoa which do lead an active life in soils (as indicated by the dilution method) are capable of limiting the numbers of bacteria present in the latter.* But this point still requires some elucidation.

IV. THE INFLUENCE OF PROTOZOA ON THE NUMBERS OF BACTERIA DEVELOPING IN AMMONIFYING SOLUTIONS.

In order to obtain some information on the capacity of soil protozoa for destroying bacteria in solutions, it was thought necessary to have a method of suppressing the former. In the literature one finds that P. T. Müller[1] employed Saponin for this purpose, in connection with his investigations on the protozoa of swimming-baths. The concentration used was ·5 % and it is stated that this had no injurious action on the bacteria.

The use of Saponin was, therefore, applied to ammonifying solutions inoculated with soil. 1 % bloodmeal in water was heated to one and a half atmospheres in the autoclave and filtered. ·05 % K_2HPO_4 was added and 100 c.c. of the solution sterilised. After cooling this nutrient solution was inoculated with 5 grams of garden soil and incubated for 18 hours at 22° C. The bacterial content of the solution was determined (agar, incubated at 22° C., has been used as medium for bacterial counts all through this section and the results are stated in the tables as millions per c.c.). The solution was divided into two equal parts in small sterile flasks and one portion received ·5 % saponin. Plates were poured from both portions at the intervals indicated in Table 12.

The active protozoa present were counted by the microscopic method. The immediate effect of the saponin is seen in the depression in the numbers of bacteria in the solution. This, however, does not last long. After 24 hours the protozoa developing in the solution without saponin begin to exercise a decided depressing effect on the number of bacteria and this has continued throughout the experiment. But the contrast between the bacterial contents of the two portions is doubtless somewhat minimised because the saponin has failed to suppress entirely the protozoa.

[1] *Arch. f. Hyg.* Bd. 75, 1912, p. 321.

TABLE 12. *Bacterial Content of solution before division* = 2·6.

Time since division of solution	Solution with saponin		Solution without saponin	
	Bacteria (c.c.)	Protozoa (c.c.)	Bacteria (c.c.)	Protozoa (c.c.)
1 hour ..	4·55	—	6·45	—
3 hours ..	9·05	—	12·80	—
6 „ ..	12·90	—	31·00	—
24 „ ..	75·00	—	56·00	400 F
4 days ..	100·00	400 F	56·00	15,000 F 1000 C
10 „ ..	68·00	200 F and C	26·00	200 F 1200 C
20 „ ..	220·00	1200 C 400 A	23·50	Under 200
30 „ ..	260·00	—	14·90	—

F = flagellates. C = ciliates. A = amoebae.

After 10 days a clearing in the saponin solution set in which, taken in conjunction with the great increase in the bacterial content, appears to point to the decomposition of the saponin. In order to test this two equal quantities of filtered 1 % bloodmeal solution + K_2HPO_4, one of which contained ·5 % saponin, were each inoculated with 1 c.c. of a protozoa-free culture of bacteria. Bacterial counts were made at the intervals shown in Table 13.

TABLE 13.

Time since inoculation of solutions	Bacterial content of solution	
	With Saponin	Without Saponin
1 day	185	185
4 days	1250	1700
10 „	2300	1050
20 „	1600	650

From the results here obtained it is very probable that the bacteria attack the saponin and that the resulting increase in bacterial numbers will exaggerate the destructive effect of the protozoa. A second disadvantage in the use of saponin is that at a concentration of ·5 % it does not entirely suppress the protozoa. Higher concentrations have been tried but up to 3 % one can never be certain that the whole of the

protozoa will be excluded. It appears, therefore, that saponin is of little value for this purpose and its use has been abandoned. In the work described by P. T. Müller the action of the saponin was quite satisfactory. But it must be noted that water was employed as the medium, not a nutrient solution.

Recourse was next had to the simple method of inoculation of the solutions with bacteria alone and with bacteria + protozoa. 50 c.c. quantities of 1 % bloodmeal solution (filtered) + ·05 % K_2HPO_4 were employed. One flask was inoculated with bacteria + protozoa from a culture of protozoa from soil, the other received as nearly as possible an equal inoculation from the same culture of bacteria alone. The method of inoculation was the single drop method already referred to. Table 14 shows the numbers of bacteria and protozoa developing in the solution.

TABLE 14.

Time since inoculation of solution (days)	A			B		
	Bacteria alone	Bacteria + Protozoa		Bacteria alone	Bacteria + Protozoa	
		Bacteria	Protozoa		Bacteria	Protozoa
1	10	8	—	Fewer than ·01	·03	—
6	736	505	65,000 F	860	801	—
10	625	350	25,000 F	2100	1400	C under 200
20	700	270	15,000 F	1120	49	1600 C
30	370	53	25,000 F	635	21	200 C

It will be observed that in both experiments the solutions to be compared started with practically equal inoculations of bacteria and that the subsequent depression in the bacterial numbers is marked and runs more or less parallel with the numbers of active protozoa present. In Experiment B, after 20 days in addition to the 1600 ciliates given at least 50,000 cysts were counted. This accounts for the very rapid fall in the number of bacteria between the tenth and twentieth days. The results after 30 days indicate very clearly the destructive power of the protozoa. In A, flagellates only were present; in B ciliates only, and as was to be expected the results show that the latter are the more active in the killing off of the bacteria.

This method of inoculation, although it has given quite good results, is not entirely satisfactory. The difficulty lies in the uncertainty as to whether the protozoa will develop after inoculation. This is probably due to the fact that the inoculum is very small compared with the bulk of the medium. The protozoa are thus forced to encyst until the bacteria develop and during this process the bacteria very frequently appear to take the upper hand.

Another method of inoculation was tried. The medium (·4 % bloodmeal, unfiltered, + ·05 % K_2HPO_4 in 100 c.c. quantities in Erlenmeyer flasks) was inoculated from a protozoa-free bloodmeal culture, each flask receiving a loopful. After two days at 22° C. some of the flasks received in addition a loopful of a bloodmeal culture containing protozoa from soil, so that from the beginning they contained more bacteria than the protozoa-free cultures. The development of the protozoa was now much more regular. Bacterial counts were made after 10 and 20 days and the numbers of active protozoa were determined roughly by the microscopic method. The results are shown in Table 15.

TABLE 15.

No. of Expt.	After 10 days			After 20 days		
	Bacteria alone	Bacteria + Protozoa		Bacteria alone	Bacteria + Protozoa	
		Bacteria	Protozoa		Bacteria	Protozoa
1	480	260	30,000 F	167	156	10,000 F
2	790	420	5,000 F	260	358	0
3	530	440	5,000 F	510	235	All encysted
4		600	10,000 F		320	All encysted
5	870	480	25,000 F	420	90	60,000 F
6		780	20,000 A		150	All encysted

Quite a marked reduction in the bacterial numbers is obtained as a result of the presence of the protozoa in all six experiments. The reduction is, however, somewhat variable and even varies during the course of the individual experiments. In 2, for example, although the protozoa have caused a great reduction in the numbers of bacteria after 10 days, after 20 days the number of bacteria in the protozoa culture is actually higher than in the protozoa-free culture. The protozoa present in this case were large flagellates. But after 20 days

no traces of protozoa, active or encysted, could be found. The protozoa had probably died off without encysting and then been attacked by the bacteria. This view receives support from the frequent observation in ammonifying solutions of protozoa, showing absolutely no signs of life but yet without any traces of a cyst membrane surrounding them. It is quite probable, therefore, that some species of protozoa die off without being able to encyst when the concentration of ammonia or other products of the activity of bacteria reaches a particular level. Their bodies would then be a ready prey to the attacks of bacteria and the latter might increase in numbers as a consequence.

The reductions in the numbers of bacteria as obtained in these experiments are on the average smaller than those given in Table 14. But it must be remembered that the bacterial content of the protozoa cultures at the beginning was in all cases larger, probably often much larger, than that of the protozoa-free culture. The only satisfactory method for securing comparable results, therefore, is the inoculation of equal numbers of bacteria from a protozoa culture in the one instance and from a protozoa-free culture (prepared from the protozoa culture) in the other, on to fresh media and the determination of bacterial numbers in both solutions at intervals.

The results given in this section prove conclusively that the soil protozoa, in solutions at all events, exercise a very decided limiting effect on the numbers of bacteria. The question of the relative activity in this direction of the three main groups of protozoa—flagellates, ciliates and amoebae—remains to be investigated.

V. THE INFLUENCE OF PROTOZOA ON AMMONIFICATION IN SOLUTION TESTS.

As a preliminary experiment in this direction, the quantities of ammonia produced in some of the cultures used in the last section were determined. The conditions in these cultures may be briefly recapitulated. Each culture contained ·4 grm. bloodmeal + ·05 grm. K_2HPO_4 in 100 c.c. water. After sterilisation in the autoclave at two atmospheres pressure, each was inoculated with one loopful of a protozoa-free bloodmeal culture and incubated for two days at 22° C. Some of the cultures then received each one loopful of a bloodmeal protozoa culture from soil. At the end of the incubation period (20 days at 22° C.) all were distilled with magnesia and the ammonia

evolved determined (see Table 16, which gives the results after deduction of controls).

TABLE 16.

Number of Experiment	Mgs. nitrogen as ammonia in culture containing:—	
	Bacteria alone	Bacteria + Protozoa
1	21·4	21·3
2	20·6	19·4
3	19·6	17·5
4		18·3
5	19·7	18·0
6		19·0

From the results of the bacterial counts (Table 15) one would naturally expect that ammonification would be depressed in presence of the protozoa. But the protozoa cultures have given an ammonification figure only slightly lower than that obtained in the protozoa-free cultures. The difference is comparatively insignificant. When the conditions prevailing in these experiments are kept in mind it seems probable that the higher original bacterial content of the protozoa cultures may account for the unexpectedly high ammonification number obtained from them. It is probable that the ammonification in the protozoa cultures, before development of the latter organisms, may have been very rapid—so rapid that the subsequent fall in bacterial numbers and consequent ammonifying power has been only just capable of neutralising it.

The only satisfactory method of deciding the matter seemed to be the inoculation of equal numbers of bacteria into solutions with and without protozoa. The microscopic method of counting bacteria was employed for this purpose. But in the case of these bloodmeal solutions the method was rather uncertain in its results, because of the difficulty in distinguishing the smaller species of bacteria from fine particles of bloodmeal, etc. The numbers of bacteria counted in the solutions, as a result of plating on agar, showed wide differences from those given by the microscopic method. In the first set of experiments the solutions were inoculated from bloodmeal cultures of protozoa + bacteria and bacteria alone, respectively. The inoculations of bacteria were arranged by the microscopic counting method so as to be approximately equal. The counts on agar at 22° C. indicated, however, that the

protozoa-free cultures had each received about 353 millions, the protozoa cultures on the other hand 440 millions of bacteria. The solutions employed were similar to those used in the last experiment. It was found advantageous to incubate all the cultures for two days with equal inoculations of protozoa-free culture before inoculation with bacteria or bacteria + protozoa as the case might be. The solutions were incubated for a total period of 20 days, from the first inoculation, at 22° C. The protozoa were present in observable numbers in two days after inoculation—*i.e.* four days from the first inoculation with bacteria. The ammonia was determined by distillation with magnesia and the results so obtained (after deduction of controls) are shown in Table 17.

TABLE 17.

Number of Experiment	Mgs. nitrogen as ammonia in solution containing:—	
	Bacteria alone	Bacteria + Protozoa
1	19·3	15·3
2		16·4
3	21·2	14·3
4		15·2
5		15·7

In spite of the fact that the protozoa cultures started out with an inoculation of 87 millions or about $\frac{1}{4}$ more bacteria than the protozoa-free cultures, they give a markedly lower figure for ammonification. The averages are, for the protozoa-free cultures 20·3 mg. N, and for the protozoa cultures 15·4 mg. N. This difference lies well outside the limits of experimental error.

In the last experiment which it has been possible to carry out in this direction, the bloodmeal cultures were inoculated, as above described, with 580 millions bacteria and 480 millions bacteria + protozoa (as indicated by counts on agar at 22° C.). The conditions were otherwise the same as in the previous experiment. The quantities of ammonia produced in the cultures after 20 days at 22° C. are shown in Table 18. (Controls have been deducted.)

It is unfortunate that in this case the original inoculation of bacteria in the bacteria + protozoa cultures was so much smaller than that in the bacterial cultures. The experiment is, therefore, of little value in helping towards a solution of the question.

TABLE 18.

Number of Experiment	Mgs. nitrogen as ammonia in solution containing:—	
	Bacteria alone	Bacteria + Protozoa
1	19·5	16·2
2		17·3
3	19·6	16·9
4		16·0

As to the appearance of the cultures with and without protozoa the latter have generally been somewhat brown in colour, the former greenish. Further the two sets of solutions smell quite differently. In the protozoa cultures the vile-smelling decomposition products usually associated with ammonification appear to be absent.

It had been intended to carry this section of the work much further but circumstances unfortunately do not permit. The results, so far as obtained, do not justify any very definite conclusions. The organisms dealt with are, with one exception, the flagellates, and it seems probable that these may have a depressing influence on ammonification. The whole question, however, requires to be thoroughly investigated.

VI. THE INOCULATION OF PROTOZOA INTO PARTIALLY STERILISED SOILS.

In the second paper of Russell and Hutchinson[1] on the effect of partial sterilisation of soils, it is stated that the authors have failed to observe a depression in the numbers of bacteria in partially sterilised soils as a result of inoculation with mass cultures of protozoa. This is attributed to the great multiplication of bacteria which takes place on the introduction of the considerable quantity of nutrient material contained in the culture. Greig Smith[2] also failed to obtain a reduction in the numbers of bacteria, after inoculation of partially sterilised soil with protozoa cultures.

Two experiments bearing on this point have been carried out here. For the first experiment 500 grams of air-dry soil was passed through a 2 mm. sieve. 2·5 c.c. formalin in 20 c.c. water was rubbed up with

[1] *Journ. of Agric. Sc.* v. 2, p. 152.

[2] *Proc. Linn. Soc. N. S. Wales*, Abstracts, 1912, pp. 2–3; Ref. *Centralbl. f. Bakt.* Abt. II. Bd. 39, p. 152.

the soil in a mortar and allowed to act in a glass bottle with close-fitting stopper for six days. A sterile suspension of 3 grams freshly slaked lime in 50 c.c. water was added to combine with the formalin and render it harmless. The bottle was placed in the 38° C. incubator for one day. After some weeks at room temperature the soil was thoroughly broken up with a large, sterile, metal spatula and weighed out in 20 gram quantities into sterile Erlenmeyer flasks. The water content was not determined but it probably amounted to about 10 %.

In order to try to minimise, as much as possible, the effects of the nutrient matter in the protozoa culture solution, soil extract + ·05 % K_2HPO_4 was selected as medium. This was inoculated with soil and after the protozoa had developed a protozoa-free culture was prepared from it. Both soil extract cultures were kept for about two months before being used for inoculation purposes. Two of the flasks containing sterilised soil received each 1 c.c. of the protozoa culture, the other two 1 c.c. of protozoa-free culture. All four received 1 c.c. of sterile water each, in addition, in order to bring up the water content of the soil to about 20 % (roughly 70 % of the water-holding capacity). In order to represent, more or less, the conditions obtaining in Russell and Hutchinson's experiments a second series of four flasks was inoculated, two with protozoa + bacteria and two with bacteria alone as in the last case. The sterile water was replaced in this instance by an equal quantity of a sterile 2 % filtered fleshmeal solution. Of the controls two received 2 c.c. sterile water each, the remaining two each 1 c.c. sterile water and 1 c.c. sterile fleshmeal solution.

The bacterial content of the protozoa-free culture was 121 millions per c.c.: that of the protozoa culture 12 millions per c.c. (agar at 22° C. was used as medium for the counts in this section). The numbers of bacteria in the soil samples used in the experiment were determined after 20 days at 22° C. The water contents were adjusted once more to roughly 20 % with sterile water and the flasks were allowed to remain for a further period of 20 days at 22° C. The bacterial contents of the soil samples were again determined (Table 19).

The results of the bacterial counts are rather irregular. This is probably due to the fact that the soil samples used were only watered once during the experiment. The inoculation of bacteria, therefore, probably did not get thoroughly distributed in the soil. The only cultures which have shown a decided depression in bacterial numbers after 40 days (as compared with 20) are Nos. 7 and 8. Here the lowering in numbers is quite marked and considerably larger than in any other

case. After the bacterial counts were made the soil samples were covered with soil extract + K_2HPO_4 and incubated for seven days at 22° C. At the end of this period the cultures so made from Nos. 3, 4, 7 and 8 contained active protozoa. Nos. 7 and 8 showed decidedly larger numbers than did 3 and 4. The remaining four soil samples as well as the controls showed no protozoa. But the original "sterilised" soil and the controls contained numerous bacteria.

TABLE 19.

No.	Inoculation	Bacterial content (millions per gram) after	
		20 days	40 days
1	1 c.c. protozoa-free culture	155	100
2	+ 1 c.c. sterile water	240	240
3	1 c.c. protozoa culture	180	200
4	+ 1 c.c. sterile water	110	160
5	1 c.c. protozoa-free culture	170	250
6	+ 1 c.c. sterile fleshmeal soln.	255	220
7	1 c.c. protozoa culture	310	200
8	+ 1 c.c. sterile fleshmeal soln.	340	140

From the results here given it is probable that the inoculated protozoa have been active in Nos. 7 and 8. But the period of activity under the conditions of the experiment must have been a short one, as after the single watering the soil would very soon become too dry for active life. This, in all probability, accounts for the comparatively small depression in bacterial numbers.

For the confirmatory experiment the soil was sterilised with formalin in the flasks in which it was to be subsequently used. Quantities of 50 grams of air-dry sieved soil were rubbed up in a mortar with 2 c.c. of a solution containing 5 c.c. formalin + 35 c.c. water. Forty-five grams of the soil was immediately weighed out into each of the flasks. The flasks used were small Erlenmeyers closed by tight-fitting corks. The formalin was allowed to act for six days and was then decomposed with slaked lime as described in the last experiment. Each flask received 5 c.c. of a sterile suspension of 5 grams $Ca(OH)_2$ in 100 c.c. water (water content of soil in flasks = 70 % W.H.C.). The flasks were placed in the 38° C. incubator for 24 hours. The soil in each was thoroughly broken up with a sterile spatula and the flasks put back in the incubator for another day. The corks were then replaced by sterile cotton-wool stoppers and the flasks weighed. After several

days in the 38° C. incubator to hasten evaporation, the flasks received the inoculations shown in Table 20 and the water content of the soil was brought up to 70 % W.H.C. The water content was readjusted once a week to this level and after 25 days bacterial counts were made for the various soil samples.

TABLE 20.

No.	Inoculation	Bacterial Content (millions per gram)
1	1 c.c. protozoa-free culture	100
2	+ 1 c.c. protozoa culture + 1 c.c. sterile water	52
3	1 c.c. protozoa-free culture	133
4	+ 1 c.c. protozoa culture + 1 c.c. sterile 2 % fleshmeal solution	77
5	1 c.c. protozoa-free culture	—
6	+ 2 c.c. sterile water	860
7	1 c.c. protozoa-free culture	420
8	+ 1 c.c. sterile water + 1 c.c. sterile fleshmeal solution	950
9	2 c.c. sterile water + 1 c.c. sterile fleshmeal solution	—
10	3 c.c. sterile water	—

Soil extract cultures were prepared from the soil samples as in the last experiment. Those from Nos. 1–4 showed numerous active flagellates after seven days at 22° C. In the remainder of the cultures no protozoa were found. The controls 9 and 10 remained practically sterile. They contained fewer than 10 bacteria per gram. The plates poured for No. 5 remained sterile. The lowest dilution used was one million. It is practically certain, however, that this must have been due to a slip in the manipulation, and as the samples had been used for soil extract cultures before it was discovered, the mistake could not be rectified. At all events the soil extract culture showed quite as good a development of bacteria as was got from samples 6, 7 and 8.

The protozoa-free culture contained 184 millions, the protozoa culture 24 millions bacteria per c.c. and as the soils inoculated with protozoa received in addition 1 c.c. of the protozoa-free culture they contained at the beginning of the experiment about 24 millions more bacteria than the soils inoculated with protozoa-free culture alone. But during the course of the experiment the conditions have become reversed and the soils containing protozoa now show a bacterial content of, on the average, about $\frac{1}{8}$ that of the soils inoculated with protozoa-free culture. *The reduction in bacterial numbers in the soils inoculated*

with protozoa is very marked and lies well outside the limits of experimental error. The conclusion may safely be drawn, therefore, that the limiting factor or at least one limiting factor (of Russell and Hutchinson) has been inoculated into the sterilised soils and has produced its effects on the numbers of bacteria. This limiting factor can thus be cultivated on soil extract medium. That it has not simply been introduced into the sterilised soils with the soil used for inoculation of the soil extract (*i.e.* without having grown on the latter) is proved by the fact that for the second experiment sub-cultures (made by inoculation of one loopful of the original cultures on to fresh sterile medium) were used. Large numbers of protozoa were observed in the solutions used for inoculation and these organisms were cultivated once more on soil extract from the soils which showed low bacterial counts. And, as it has been shown that the protozoa are capable of reducing the numbers of bacteria in solutions, it appears justifiable to consider them as the limiting factor in soils.

In conclusion I wish to thank Prof. Löhnis for having suggested this work on the soil protozoa and for advice, ever at my disposal, during the carrying out of it.

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STUDIES ON THE LIME REQUIREMENTS OF CERTAIN SOILS.

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(With Plate I and 4 text-figures.)

The fertility of a soil may be stated to depend on two functions, namely, its capacity to supply the necessary nutrients for plant growth, either by virtue of original reserves or by biological action, and its suitability as a matrix, serving to hold plant roots and possessing definite relations to air, water and temperature; these determine very largely the amount of growth attained by any crop. Since, however, the growth of soil organisms as well as that of any introduced plants is very sensitive to the reaction of the matrix, it follows that cases may arise in which the presence or absence of a base would definitely set a limit on crop production.

The compounds commonly used to correct soil reaction in the field are those of calcium—either as oxide, hydroxide, or carbonate, as in chalk, limestone, or marl; the benefits accruing from their use are well recognised, but the difference in their type of action has not hitherto received the attention it deserves.

In two earlier communications it has been shown that caustic lime exercises a specific effect on the soil, producing when applied in sufficient quantity certain effects common to those which have been classed under the head of "partial sterilisation." Calcium carbonate does not exercise this action, but either form may be used for correcting soil reaction. In practice this difference has not been recognised and has been responsible in many instances for the misinterpretation of experimental work, since it is difficult to determine precisely how far any result has been due to neutralisation or to sterilisation effects.

It is necessary therefore in any particular case to decide firstly on the type of change to be induced (sterilisation or neutralisation), and secondly, the amount of lime required to bring about the desired effect. The first consideration can only be decided by reference to the particular conditions of each case, while guidance as to the second is afforded by the two methods suggested below for the two distinct purposes. We propose accordingly to divide the paper into two sections, viz.,

Part I. The Determination of the Amount of Lime (CaO) necessary to induce Partial Sterilisation Changes.

Part II. The Determination of the Amount of Lime (CaO) or Chalk ($CaCO_3$) required for Soil Neutralisation[1].

Part I. Lime Requirements for Partial Sterilisation Purposes.

The capacity of caustic lime to induce typical partial sterilisation changes has been studied with a number of soils and the results of these bacteriological, chemical and pot-culture observations have already been given in detail elsewhere (5). The results thus obtained serve as a check on those given by the method below.

The addition to the soil of any partial sterilisation agent results in changes of which the three chief are (*a*) an initial reduction in the numbers of putrefactive bacteria, with a subsequent increase in numbers and activity; (*b*) the partial or complete inhibition of nitrifying organisms, thus leading to accumulation of the ammonia produced by break-down processes, and (*c*) a similar inhibition of the larger soil protozoa. With the two latter, somewhat related effects on "soil pests" might possibly be associated.

During this work it was evident that the various soils differed greatly in response to treatment and that the conventional methods failed to give any index to this relative responsiveness. Since the desired changes, chiefly of a biological nature, could only be due to an alteration in the reaction of the soil solution, it was obviously necessary to obtain some gauge of the absorptive power of the soil for lime.

[1] This term is used with due reservation. Although the view has been advanced by van Bemmelen (1) and Baumann and Gully (2) that the phenomena of "soil acidity" may be explained on a purely physical basis, this has been severely criticised by Rindell (3) and Tacke and Süchting (4) and the authors consider that it would be somewhat premature to abandon the term "acidity." It is therefore used in these pages as convenient to indicate "apparent acidity," "lack of basicity," or in this case "lime requirement"; "neutralisation" may be interpreted as the correction of this condition.

Some observations on this question were made by Way (6), who employed lime water, and further work might naturally proceed along these lines, or by adding solid calcium oxide in varying quantities to the moist soil to be tested. The latter method promised, however, to approach more closely to the conditions obtaining in our other bacteriological and chemical experiments with soil in bottles, and was accordingly adopted. We confined ourselves in the first instance to the study of some five soils—Rothamsted, Millbrook, Woburn, Chelsea and Craibstone—which had been studied in other directions.

The Method. The method originally adopted and to which we have adhered throughout is based on the determination of the minimum amount of lime required to render the soil water distinctly alkaline and is as follows: 100 grm. lots of the air-dry soil to be tested are placed in bottles of about 250 c.c. capacity; according to the character of the soil (whether poor or rich, light or heavy) a number of dressings of calcium oxide are then made, rising by increments of 0·1 grm. to 1·0 per cent., or increments of 0·2 grm. to 2·0 per cent. of the weight of soil. Sufficient water (50 c.c.) is added to moisten the soil, the bottles are then tightly corked and shaken for a few seconds at intervals for a definite period. This period is generally 24 hours, but actual comparisons have shown that the amount of change between 4 and 24 hours is only slight. At the end of this time the contents of the bottles are then transferred to, and washed in, a Buchner funnel with a further 200 c.c. of water; the whole of the filtrate is then titrated with N/10 acid, using phenolphthalein as indicator.

Within the range of applications made in the above manner it will generally be found that a point is reached where the reaction of the filtrate is distinctly alkaline, and the results of other investigations have shown that where the alkalinity is such that 5–10 c.c. of N/10 acid are required to neutralise the whole of the filtrate, this may be taken as the limit to which calcium oxide must be applied to the soil in order to produce the best results.

With heavier applications the concentration of the filtrate tends to approach saturation point, but any increase in the application beyond the amount necessary to bring the filtrate to the above-mentioned point appears undesirable; such applications tend to constitute "over-liming" if made under ordinary conditions. The results of a few such tests are given in Table I, and are plotted, together with data obtained with other soils, in Curve 1.

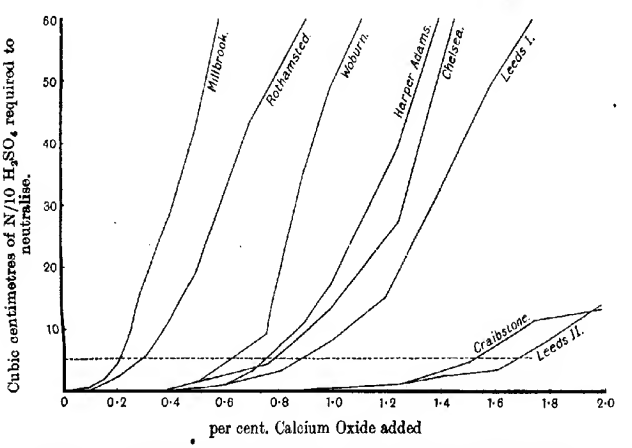
The great difference in absorptive capacity of the various soils is

apparent, while the order in which these are ranged possesses certain points of interest that may be noted here.

TABLE I. *The Determination of the Partial Sterilisation Point by the Titration Method.*

(The results are expressed as c.c. N/10 acid required to neutralise the whole of the filtrate.)

Rothamsted		Millbrook		Woburn		Chelsea		Craibstone	
CaO added	Titrn. c.c.	CaO added	Titrn. c.c.	CaO added	Titrn. c.c.	CaO added	Titrn. c.c.	CaO added	Titrn. c.c.
%		%		%		%		%	
0·1	0·5	0·1	0·5	0·50	2·0	0·25	0·9	0·25	0
0·2	2·2	0·2	4·5	0·75	9·5	0·50	1·8	0·50	0
0·3	5·6	0·3	16·6	0·90	35·0	0·75	4·6	0·75	0·1
0·4	11·5	0·4	27·6	1·00	48·9	1·00	13·4	1·00	0·6
0·5	18·2	0·5	42·1	1·25	75·4	1·25	27·2	1·25	1·2
0·6	26·9	0·6	63·5	1·50	84·2	1·50	66·8	1·50	4·6
1·0	67·3	1·0	95·3	2·00	93·0	2·00	88·9	2·00	12·8



Curve 1. The Determination of the Amount of Caustic Lime required to induce Partial Sterilisation of a Soil.

The addition to any soil of the amount of lime indicated by this titration method is sufficient to affect appreciably the reaction of the soil water and hence may be expected to displace more or less

radically the biological conditions obtaining therein. This we find to be the case with all the soils over which an adequate control has been exercised. For purposes of comparison the relations of the nitrifying organisms and the larger soil protozoa towards treatment are given in the following table.

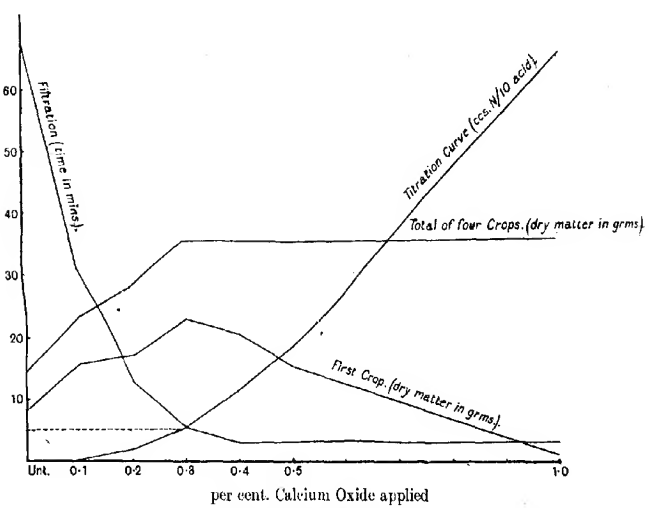
TABLE II. *The Relation between the Critical Point indicated by Titration Method and the Amounts of Lime necessary to induce certain changes in various Soils.*

	Rothamsted	Millbrook	Woburn	Chelsea	Craibstone
Critical point indicated by titration method	0·3	0·2	0·6	0·75	1·0
Inhibition of nitrification (laboratory experiment)	0·3	0·3	0·5–1·0	0·5–1·0	1·0
Destruction of larger protozoa (laboratory experiment)	0·3–0·4	0·2–0·3	0·5–1·0	0·5–1·0	1·0
Maximum growth of 1st crop (pot experiment)	0·3	0·3	0·6	0·2	1·0
Maximum growth of first four crops	0·3	0·3	—	1·0	1·0

The titration method therefore allows of a good approximation of the probable biological changes resulting from treatment of a soil with a given quantity of caustic lime. With respect to the crop producing power of treated soils the conditions are somewhat more complicated. Laboratory work has shown that the returns of nitrogen as ammonia and nitrate are fairly proportional to the amount of lime applied (i.e. within the first 9–12 months), but in heavily limed soils this nitrogen is largely in the form of ammonia. Consequently, plants growing in such soils obtain their nitrogen in a non-nitrate form and utilise it, as has been frequently demonstrated, in an uneconomical manner; hence no direct relation exists between the total ammonia and nitrate produced in two differently limed soils—in the one case where the nitrogen is chiefly in the form of nitrate, and in the other in the form of ammonia. The pot experiments carried out with the above soils have served to show, however, that an application to a soil of an amount of caustic lime equal to that indicated by the titration method results (*a*) in a maximum production of dry matter in the first crop—heavier applications tending to give a relative or actual depression, thus constituting "over-liming," and (*b*) in a maximum growth of the four crops used in our experiments. Hence the method proposed may serve as

a safeguard against (1) "over-liming" and its undesirable consequences, and (2) the uneconomical utilisation of soil nitrogen.

When the application of lime is sufficient to impart an alkaline reaction to the soil water, certain other changes appear to be induced. During a number of titration tests with various soils, it was clearly evident that a flocculation of the soil compounds occurred when a slight excess of lime was present, and the time required for the passage of a definite amount of wash water (200 c.c.) approached a minimum as the partial sterilisation region was reached. This was so noticeable that some readings were taken, and these are plotted along with the titration curve and dry matter production in Curve 2. These data apply to Rothamsted soil, but similar results were also obtained with Millbrook soil. Thus a close agreement obtains between the results of nitrate determinations, tests for protozoa, pot culture experiments and the titration method suggested.



Curve 2. Showing the Effect of an Application of the Critical Dose of Calcium Oxide (as indicated by the Titration Method for determining the Partial Sterilisation Point) on Filtration and on Crop Production. (Rothamsted Soil.)

PART II. LIME REQUIREMENTS FOR NEUTRALISATION PURPOSES.

By far the most frequent purpose for which lime is employed in agricultural practice is the correction of "soil acidity," whether this is due to the presence of purely mineral soil constituents, to the application of acid artificial fertilisers, or to the accumulation of organic residues evidenced by more or less pronounced peat deposits. The condition is indicated in the field by the occurrence of certain "calcifugous" plants, e.g. *Rumex acetosella, Chrysanthemum segetum, Spergula arvensis,* and *Scleranthus annuus,* by the repeated failure of leguminous crops such as clover and lucerne, or by the incidence of "finger and toe" in cruciferous crops. Declining fertility of the soil gradually becomes evident, although this is subject to variation with the different crops—such crops as barley, wheat and clover being more, and oats, rye, millet, buckwheat and potatoes less sensitive, to soil acidity.

In the laboratory various tests have been employed for indicating the desirability of lime applications, and these may be divided into two classes according as to whether they indicated (*a*) the presence of a sufficiency of bases, usually calcium, or (*b*) lack of bases or soil acidity.

General Methods for indicating Lime Reserves in the Soil.

Of the various methods designed to indicate the presence of an adequate supply of base, those for the determination of free carbonates have been most generally adopted. These methods have, however, often differed greatly in procedure and the results do not admit of direct comparison in the majority of cases. Neglecting this fact, certain standard carbonate contents have been suggested, but the evidence in favour of these is very meagre, resting as it does chiefly on the observed fact that soils containing 0·5–1·0 per cent. calcium carbonate are generally fertile, other conditions being normal, although closer inquiry shows that many soils are devoid of carbonate and are still quite productive. The method has the advantage, however, of indicating the amount of lime present in a form capable of limiting soil acidity, whilst this can by no means be claimed for other methods where the amount of lime extractable by various solvents is calculated as carbonate. Hollemann (7) proposed extraction of the soil with water saturated with carbon dioxide and estimation of the lime removed; where the amount of lime thus extractable from a clay soil fell below 0·15 per cent. (CaO) a benefit might be expected from liming.

Immendorff (8) employed a method for indicating lime reserves in the soil, in which 2·5–10 grms. of the soil are boiled for half an hour with 200 c.c. of distilled water and 25 c.c. of sulphuric acid (N/5), the liquid being then filtered and a back-titration made. Where it is a question of ascertaining the amount of easily soluble alkaline earths irrespective as to whether these are present as carbonates or silicates the method is said to give useful results.

Sestini (9) suggests the use of boiling acetic acid and Mayer (10) also used 1: 2 acetic acid on account of its inactivity towards ferrous carbonate. Extraction with hot dilute hydrochloric acid has been employed, and Heinrich (11) gives the following scale of minimum contents of lime (CaO) extractable with 10 per cent. acid. According to this a lime content of

0·05 % is sufficient for the growth of potatoes and rye,
0·05–0·10 is sufficient for the growth of oats and barley,
0·10 is sufficient for the growth of peas and vetches,
0·12 is sufficient for the growth of clover and
0·20–0·30 is sufficient for the growth of lucerne.

Maercker (12) gives two scales—one for sandy soils and another for loams—10 % hydrochloric acid being used, while Orth (13) gives still another for data obtained by the use of strong acid. It soon became evident that the lime thus removed from the soil could not fairly represent the amounts of lime available either for plant growth or for the correction of soil acidity, and other methods depending on the interaction of lime compounds in the soil with various neutral salts were suggested.

The one proposed by Stützer and Hartleb (14) rests upon the decomposition of soil carbonates by the addition of ammonium chloride, the ammonium carbonate formed being then estimated by distillation.

Following a method indicated by Kellner (15) for the determination of the absorptive power of soils, Meyer (16) elaborated one for the estimation of lime in an available state. This consists in the treatment of 10 grms. of soil with 100 c.c. of a 10 per cent. solution of ammonium chloride for three hours and the determination of the lime removed in the solution. Gregoire showed, however, that this period was not sufficient for the removal of the whole of the available lime and recommends extension of the period to 10 hours. In view of the fact that this method often gives anomalous results, Weibull (17) drew into consideration another factor—that of the organic matter of the soil as indicated by "loss on ignition." On the assumption that the lime

(i.e. soluble in ammonium chloride solution) tends to react as an alkali, and the "loss on ignition" portion acts as an acid component there should obtain a definite ratio between these two for the production of a neutral soil reaction. According to Weibull, it may be stated that (1) ordinary soils with 3–6 % "loss on ignition" and less than 0·30 % soluble lime are acid in character and possess low nitrifying power; soils poor in lime and with a more neutral reaction have a lower "loss on ignition" content.

Soils with traces or more than traces of calcium carbonate, or soils without carbonate but with more than 0·25 % available lime, are, as a rule, alkaline and possess a marked nitrifying power; as exceptions are soils with high humus content. If the ratio lime: "loss on ignition" fell below 1 : 20 the soils examined were acid in character; if above 1 : 20 the reaction was alkaline. Meyer examined a number of soils in this respect and found good agreement on the whole between the lime content : loss on ignition ratio and the reaction of the soil.

TABLE III. *Relation between the Lime Requirements of some Danish Soils and the percentage of Lime soluble in Ammonium Chloride Solution (Christensen and Larsen).*

Percentage of lime (CaO)	No. of experiments	Results of field experiments Lime response			
		positive	negative	doubtful	none and doubtful
0—0·05	16	87 %	6 %	6 %	13 %
0·06—0·10	16	87	6	6	13
0·11—0·15	17	65	24	12	35
0·16—0·20	15	40	40	20	60
0·21—0·25	17	24	65	12	76
0·26—0·30	8	0	88	13	100
over 0·30	27	11	81	8	89

His analytical data are, however, difficult of interpretation—one soil possessing 0·372 % soluble lime (CaO), 0·126 % CO_2 (= 0·286 % $CaCO_3$), an acidity of 0·054 % (expressed as CO_2) and an acid reaction, but did not respond in pot culture to an application of calcium carbonate. Of the six soils with which Meyer worked three possessed a lime content above the normal (0·25 %), five are stated to be acid in character, but only one was found to respond to carbonate applications. Briefly, the analytical data failed to throw any light on the need of lime. In connection with an extensive scheme of liming experiments initiated by the

Danish "Lime Committee," Christensen and Larsen correlated the results of the examination of a number of soils by this and other methods with the results of field trials. Those obtained with the ammonium chloride method are given below.

The probable "lime requirement" limit is thus about 0·16–0·20 %, but of the total number of cases there remain about 40 % in which little guidance is given by the method.

The Determination of Soil "Acidity."

(*a*) *Qualitative Methods.*

The simple and commonly adopted test for soil acidity is that with litmus paper, in which a strip of neutral paper is interposed for upwards of 15 minutes between two masses of the moist soil to be tested. For rough work in the field the method possesses a certain amount of value—the production of a red tint generally being an indication of acid soil conditions. On the other hand, however, the failure to give any colour change does not necessarily mean that the soil would not respond to an application of lime. The Craibstone soil largely used in our experiments failed to react to this test, but responded distinctly to treatment with carbonate both in laboratory and pot culture experiments. A refinement of the method has been introduced by Christensen and Larsen (18), who used neutral litmus solution and classified the soils according to the tint produced in the test. In the test, 1 c.c. of a neutral litmus solution and 20 c.c. of distilled water are placed in test tubes of about 40 c.c. capacity; 5 grms. of soil are then added, shaken up, and allowed to stand until the following day. According to the tint produced, the soils may be grouped into acid, weakly acid, neutral and alkaline types. Of the soils tested by these investigators, 26 were found to be acid or weakly acid in reaction and of these only one failed to respond to treatment in the field; of 50 soils found to give a neutral reaction, 58 per cent. still responded to treatment and 14 per cent. were doubtful, thus supporting the view expressed above with regard to this test.

Loew (19) employed a method based on the liberation of iodine from potassium iodide added to the soil and the production of a blue colour with starch, while Daikuhara (20) suggests the following test: 5 grms. of the soil are placed in a test tube and a 10 % solution of potassium nitrite (chemically pure, and especially free from carbonate) is added drop by drop until the soil is moderately moistened; the mouth of the

tube is then closed with a plug of cotton wool from the middle of which a strip of potassium iodide starch paper is suspended. After a short period the degree of soil acidity can be gauged by the intensity of the blue colouration produced. Another method, suggested by Baumann and Gully (21), consists in the addition of a solution containing 2·0 per cent. potassium iodide and 0·1 per cent. potassium iodate to 1–3 grm. of the soil in a test tube; after being shaken and then allowed to stand for 15 minutes the solution is filtered and an equal quantity of dilute starch solution added. The intensity of the colouration provides an index of soil acidity.

In a test proposed by Albert (22) the soil is suspended in water, a few grains of lithium phosphate added and then allowed to stand until no further colour change occurs; the tint ranges from light brown to brownish-black and depends on the formation of soluble lithium humates.

(*b*) *Quantitative Methods.*

One of the earliest methods, and one which is still largely used on the Continent, is that introduced by Tacke (23) based on the capacity of the soil to liberate carbon dioxide from finely divided calcium carbonate. The soil is placed in a flask through which a slow stream of pure hydrogen is passed to remove residual carbon dioxide from the soil and the apparatus is then opened, by means of a T-piece, to a Pettenkofer absorption tube containing N/5 or N/10 sodium hydroxide solution; an excess amount of a suspension of finely divided calcium carbonate is then allowed to flow from a separating funnel to the soil bottle. The passage of hydrogen is then continued for 2½ hours and the amount of carbon dioxide evolved is estimated by titration of the soda against phenolphthalein after the addition of barium chloride solution. The method has given satisfactory results on the whole and has the advantage over various other methods in that the change upon which it depends is the one occurring naturally in the field; its disadvantages are that the determination is somewhat wearisome and that, during the period of the determination, there proceeds a slow but appreciable degradation of organic matter with the production of carbon dioxide. To obviate this source of error, Süchting (24) has suggested the employment of a definite amount of calcium carbonate and the estimation of the residual carbonate after all change has ceased.

Wheeler, Hartwell and Sargent (25) attempted to render the Tacke method more rapid by boiling the mixture, but found that degradation

changes proceeded still more rapidly and resulted in fictitious values being obtained.

The capacity of the soil to absorb the base from neutral salts in solution has formed the basis of a number of methods. In their earlier work, Hopkins, Knox and Pettit (26) recommended that 100 grms. of dry soil be digested in the cold for three hours with 250 c.c. of a 5·0 per cent. solution of sodium chloride, the whole being shaken at intervals. At the end of this period 125 c.c. of the clear supernatant liquid was syphoned off and, after being boiled for a few minutes to drive off carbon dioxide, was titrated with standard alkali against phenolphthalein. Actual experiment showed that displacement proceeded until an equilibrium was established and the removal of the liquid and successive treatment of the same soil with fresh quantities of solution resulted in values being obtained which were in the order of decreasing geometrical progression. For routine work, however, it was found convenient to employ a factor—the results of the titration of the first 125 c.c. $\times 3$ giving results equal to those obtained by successive digestions. The factor subsequently suggested was 4, while still later (27), where a normal solution of potassium nitrate was employed, one of $2\frac{1}{2}$ was advised. The earlier method has been criticised by Daikuhara (28), who was able to show that the values given by sodium chloride were very much lower than those obtained when other—chiefly ammonium and potassium—salts were used, and suggests that potassium chloride be employed. Daikuhara's own work shows also that this salt gives values very similar to those obtained with potassium nitrate, and he does not appear to be aware that the latter salt had already been recommended by Hopkins. The factors given by Daikuhara are 3·5 and 3·0 for digestions of one and five days respectively. Employing this method, he examined a very large number of so-called "acid mineral soils," such as appear to be widely distributed throughout Japan and Korea, and the results present several points of interest. One characteristic of the soils is that the application of neutral mineral manures results in decreased crop yields, whilst the zinc pots in which the experiments were made were gradually attacked and finally perforated. The soils fail to give an acid extract with water and give a reaction with litmus paper only at the point of contact with the soil. With neutral salt solutions an acid reaction is produced immediately and this is advanced as a test for such acid soils. It is important to note, however, that of 917 Japanese virgin soils, 738 gave a response to litmus paper and only 467 reacted to this test; the method is not, therefore, generally applicable.

The detailed analysis of the potassium chloride solution after digestion with the soil had taken place showed that an interchange of bases had occurred and large quantities of alumina and iron had come into solution in the form of acid salts—in fact, the characteristic reaction of the soils is attributed to the presence of alumina[1] and iron compounds which are loosely absorbed by soil colloid bodies.

Two other methods, in which salts of weaker acids are used, have been described. According to Loew (29) 50 grms. of finely ground air-dry soil are digested with 200 c.c. of neutral 1·0 per cent. solution of sodium or potassium acetate at room temperature for 24 hours. The acetic acid is more easily displaced than the stronger mineral acids and is estimated by the titration of an aliquot portion of the filtrate.

The bases employed by Loew are obviously not such as would come into consideration for the correction of soil reaction in the field, and Jones (30) has accordingly used the calcium salt; 5·6 grms. of soil are ground in a mortar with 0·5 grm. neutral calcium acetate and then sufficient water is added to make a stiff paste. Grinding is continued and a further 30 c.c. of water added and mixed for 30 seconds; the whole is then transferred to a 200 c.c. measuring flask, the volume made up to about 160 c.c. and allowed to stand (with occasional shaking) for 15 minutes, after which water is added to make up to 200 c.c. and filtered. The first 10 or 15 c.c. of the filtrate are rejected and a further 100 c.c. taken for titration with phenolphthalein. This reading $\times 2 \times 1{\cdot}8 \times 1000$ gives the amount of lime[2] in lbs. per acre of two million pounds of soil. The method is extremely rapid, but in our own tests has given far from satisfactory results.

A further group of methods rests in the absorptive capacity of the soil for various alkaline compounds. In that of Wheeler, Hartwell and Sargent (31) the soil is digested at room temperature for 42 hours with a known volume of approximately N/10 ammonia. A portion of the clear supernatant liquid is withdrawn and hydrochloric acid added to throw down the humic acid; after filtration the excess of acid is estimated by titration. A colorimetric method was also attempted by these authors. These and similar methods involving the use of alkaline compounds are, however, open to the objection that whether the soil

[1] The unproductiveness of certain American soils has been ascribed to the presence, in the soil, of soluble aluminium salts (Abbott, Connor and Smalley, Purdue Univ. Agric. Exp. Stat., *Bull.* 170. 1913).

[2] Chalk ($CaCO_3$) is evidently intended, and not lime (CaO) as stated in the original paper.

be acid or neutral a considerable amount of the compound is directly adsorbed and we have no means of determining this amount. The experiments in the first part of this paper will suffice to demonstrate this adsorption—such neutral soils as the Rothamsted and Chelsea samples exhibit marked powers for taking up calcium hydroxide. Furthermore, where ammonia is used it is practically impossible to obtain a clear filtrate or supernatant liquid. As an alternative to Tacke's method, Albert (32) proposed one in which the absorptive capacity of the soil for calcium, magnesium or barium was determined, the latter being preferred on account of the lower dissociative power of its salts. For the determination, 20–50 grms. of the air-dry soil (according to acidity) are placed in a Jena glass flask together with 200 c.c. of distilled water; 50–100 c.c. of N/5 barium hydroxide solution are run in from a burette and 10 grms. of solid ammonium chloride added. The flask is fitted immediately to a condenser and the contents boiled for 20–25 minutes, the ammonia evolved being collected in N/10 acid and a titration made with sodium alizarin sulphonate as indicator. Since the amount of the ammonia displaced from the ammonium chloride is equivalent to the amount of barium hydroxide not absorbed by the soil, this absorption or the soil acidity may be determined. The values obtained by the use of barium, calcium or magnesium hydroxide were found to vary widely among themselves. According to Süchting and Arnd (33), Albert's method is of little value since the results are distinctly dependent on the period and intensity of boiling.

Lyon and Bizzell (34) have recently introduced a modification of Albert's method. The reaction between soil and alkali does not occur immediately and these workers recommend the addition of the barium hydroxide solution about 60 minutes before that of the ammonium chloride the soil and alkali being kept at boiling point in the meantime. The procedure allows of two sources of error; the first is due to the capacity of all soils to expel a quantity of ammonia from the ammonium chloride employed when the mixture is subjected to heat, and Lyon and Bizzell suggest a correction for this—a blank determination being made with soil and ammonium salt but without alkali. In some of our own work with this method the amount of ammonia thus expelled by a soil deficient in lime and with the addition of the prescribed quantity of ammonium chloride amounted to 20–25 per cent. of the total produced on the addition of barium hydroxide as in the standard method; the distillation of a further 50 c.c. increased this error to over 30 per cent. In the second place, all soils liberate

appreciable quantities of ammonia when heated with barium hydroxide solution at 100° and the amount thus formed must be taken into consideration.

A method which has, perhaps, been more extensively used in the United States than any other is that introduced by Veitch (35). In this, three lots of soil each of 10 grms. are weighed into platinum basins and about 50 c.c. of water added; three different volumes, say 10, 20, and 30 c.c. of standard calcium hydroxide solution are added and the basins are placed immediately on a water bath and their contents evaporated to dryness. With the aid of 100 c.c. of distilled water the soils are then transferred to Jena glass flasks and allowed to stand overnight; the reaction of about 50 c.c. of the filtered liquid is tested by boiling with phenolphthalein, the volume being reduced by boiling, if necessary, to 5 c.c. The three quantities of lime-water taken will generally suffice for orientation one of the quantities being too small and the others too large for exact neutralisation or *vice versâ*. Within the limits thus set by the trial determination, other tests are made where the amounts of lime-water vary by no more than 2 c.c.; the approximate requirement may thus be obtained.

The method is based on the fact that the slight excess of lime-water present after the neutralisation of the acid soil material is converted into carbonates or bicarbonates, the boiling solution of which gives an alkaline reaction with phenolphthalein. The strict adherence to the prescribed directions in these methods is essential—the adoption of an extended period of digestion as, for instance, in the Albert method, or failure to place the soils at once on the steam bath as in Veitch's method would probably lead to untrustworthy results.

Wheeler and his colleagues and also Veitch attempted to obtain a measure of soil acidity by digestion of the soil with lime-water in the cold. The results thus obtained by Veitch were higher than those given by his acidity method, and there appears to be no reason to doubt that the introduction of the heat factor is responsible for this difference.

Gregoire, Hendrick, Carpiaux and Germain (36) have recently investigated the action of soils on a "Kjeldahl's solution" of the following composition:

55·3 grms. potassium iodide,
14·3 ,, potassium iodate,
99·2 ,, sodium thiosulphate (cryst.) and
1000 c.c. water.

For titration against this solution a second one containing 17·0 grms. iodine and 25·0 grms. potassium iodide per litre was used. The strength of the iodide solution is determined by taking 15 c.c. of the "Kjeldahl's solution" together with 20 c.c. of N/5 hydrochloric or sulphuric acid and titrating the excess of thiosulphate with the iodine solution, the difference between the value thus found and that of a direct titration of the "Kjeldahl's solution" being the one required.

For the determination of acidity, 10 grms. of the fine soil (passing through a 1 mm. sieve) are digested in a flask with 15 c.c. of the "Kjeldahl's solution" for a prescribed period, after which the volume is made up to 110 c.c. and 100 c.c. taken for titration. The results are chiefly expressed as H ions per kilo of soil. The amount of reaction with the soil depends, in the first place, on the concentration of the solution, and the above strength is recommended; it has further been found that the change is not immediate but continues for a considerable period with all soils, although where the soil is acid the greatest amount of change takes place within the first 24 hours. The slight additional reaction between this time and the end of 15 days is common to all soils and is doubtless due to interaction with inorganic soil constituents. It must be noted that a positive reaction is given even when the soil contains large amounts of carbonate and the setting of a limit of maximum absorption for neutral soils appears desirable.

A point brought out by the results of Gregoire and his co-workers is that a fairly large proportion of soils occur that not only do not possess any lime as carbonate but also fail to give any definite reaction as to acidity; they are, in fact, just neutral and an estimation of carbonate alone would fail to give reliable information as to the lime requirements of such soils.

Experimental.

In the course of our other work on the relative action of calcium oxide and carbonate on the soil, the need was felt of a simple and accurate method for the determination of the lime requirements of these soils. In view of the fact that carbonates and bicarbonates are the chief compounds tending to maintain a neutral reaction in the field, and that any amelioration must proceed through this change, it appeared likely that a closer investigation of the action of certain carbonates on the soil might give a measure of prevailing acidity, and would possibly conform more closely to natural conditions than some

of the methods hitherto employed. Preliminary work with sodium carbonate and bicarbonate gave unsatisfactory results inasmuch as deflocculation of the clay compounds of the soil occurred and adversely affected the rate of filtration; a coloured extract difficult of titration was obtained and, furthermore, positive absorption was shown even in the case of neutral soils containing an abundance of carbonate initially. Some of these results[1] are given below.

TABLE IV. *The Absorptive Power of Soils for Sodium Carbonate.*

Soil	$CaCO_3$ content of soil	Compound employed	Absorption (stated as $CaCO_3$ required)
Rothamsted	2·6 %	Sod. carbonate	0·390 %
„	„	„ bicarbonate	0·125
Oundle	over 40 %	„ „	0·175
Woburn	nil	„ „	0·175
Craibstone	nil	„ „	0·290

In view of these unsatisfactory results recourse was then had to the use of a solution of calcium bicarbonate, and after minor modifications as to period of digestion, strength of solution, etc., this method has been used with a large number of soils under well controlled conditions.

The Method. The required solution may be prepared either by passing a current of carbon dioxide into a suspension of calcium carbonate in distilled water, or by means of a "Sparklet" or refillable soda-water syphon, for which bulbs of compressed carbon dioxide are used. The latter method is the more convenient and permits of the preparation of a saturated solution within quite a short time. A large excess of carbonate must be used (about 10 grms.) in order to provide an abundance of small particles which pass readily into solution and the syphon requires occasional gentle shaking for about 15–20 minutes. The contents may then be diluted with one-third its volume of distilled water and filtered; this will result in the formation of a solution of approximately N/50 strength.

For a determination of acidity, or lime requirement, 10–20 grms. of the soil are placed in a bottle of 500–1000 c.c. capacity together with

[1] For general convenience and purposes of comparison the data from our soil examinations are stated in terms of calcium carbonate required to bring the soil to the neutral point. This allows of direct interpretation for general field work, each 0·1 per cent. being taken as equal to 1 ton per acre of 2½ million pounds of soil. In specific cases it is advisable that the apparent specific gravity of the soil be determined.

200-300 c.c. of approximately N/50 solution of calcium bicarbonate, and the air in the bottle is displaced by a current of carbon dioxide in order to insure against possible precipitation of the calcium carbonate during the period of the determination. The bottle is then placed in a shaking machine for three hours[1], after which time it is opened, the liquid is filtered, and a portion of the filtrate equal to half the original amount of bicarbonate solution is titrated with N/10 acid using methyl orange as indicator. The difference between this final titration and that of the initial solution represents the amount of calcium carbonate absorbed, each cubic centimetre of N/10 acid being equal to 5 mgrms. of calcium carbonate.

Preliminary work with this method served to show that with neutral soils there was practically no absorption, while the presence of carbonate in a soil often resulted in an increase in the strength of the solution during the period of the determination. For all our routine work a small rotary shaker was used which gave a gentle agitation to the contents of the bottles.

The extent of interaction between soil and solution obviously depends largely on the rate at which the soil particles are broken down, and in order to ascertain the minimum time required for this action a very unkind acid Oxford clay soil was used after being passed through a 3 mm. sieve. The results are included in the following table.

Soil	Per cent. absorption after						
	1 hour	2 hours	3 hours	4 hours	6 hours	9 hours	11 hours
Ridgmont	0·38	0·45	0·40	0·43	0·43	0·43	0·44

In view of the relatively high absorption after two hours and the lack of increase after this period, even with this heavy soil, we felt justified in taking three hours as the maximum period for further work.

As might be anticipated the strength of the bicarbonate solution regulates the amount of absorption with any given soil and it appears important that, for the reaction to approach completion within the prescribed period, the concentration of the initial solution should not

[1] Occasional shaking by hand (at intervals of 20 minutes) for four hours, gives identical results.

be much below N/50 strength. The results of a comparative experiment will suffice to illustrate this.

Initial concentration of solution	N/50	N/75	N/100
Absorption (as per cent. of soil)	0·272	0·265	0·210

Adhering to this period of digestion and N/50 strength of solution the method has been tested against some of those reviewed above, our routine soils being employed. The results of some of these comparisons are given in Table V.

TABLE V. *Comparison of various Methods for determining Soil Acidity.*

Method used after	Acidity expressed as $CaCO_3$ required to neutralise the soil (per cent.)				
	Chelsea	Millbrook	Oundle	Woburn	Craibstone
Jones	0·045	0·045	0·018	0·232	0·161
Hopkins	0·012	0·006	0·002	0·244	0·030
Lyon and Bizzell	—	—	—	0·226	0·436
Veitch	—	—	—	0·204	0·407
Bicarbonate method	nil	0·020	nil	0·260	0·430

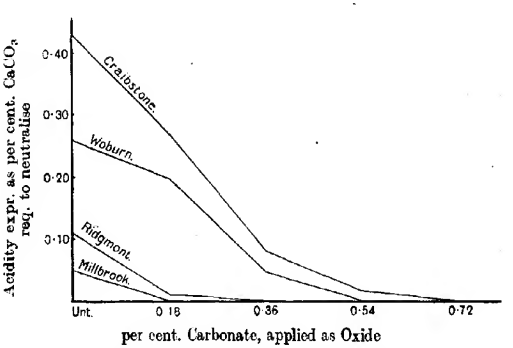
Where the soil reaction is presumably due to the presence of acid mineral compounds, as in the case of the Woburn soil, the methods proposed by Jones and Hopkins give results closely approaching those obtained by other methods, but this agreement no longer holds when the reaction or lime requirement is due to other causes, as in the Craibstone soil. It is interesting to note that in both cases the bicarbonate method gives data agreeing well with those from the more laborious Lyon and Bizzell and the Veitch tests.

In connection with routine bacteriological and chemical work, certain of the above soils had received various additions of calcium oxide and carbonate and served as control material for testing the accuracy of the method. Although the response of a soil to the correction of acid conditions can only be determined generally by carbonate applications, on account of the possible direct chemical action of the oxide on some soils, evidence will be adduced later which tends to show that so long as the neutral point is not reached, the value of oxide for neutralisation purposes is practically the same as that of carbonate. We feel justified therefore in using some of these soils to show the

reduction of acidity as indicated by the bicarbonate method. Some of the data from such determinations are given in Table VI, and plotted in Curve 3.

TABLE VI. *The Acidity of certain Soils after Treatment with Lime.*

CaO applied (stated as $CaCO_3$ per cent.)	Rothamsted	Millbrook	Woburn	Craibstone	Ridgmont
0	0	0·05	0·26	0·43	0·11
0·18	0	0	0·20	0·27	0·02
0·36	0	0	0·05	0·08	0
0·54	0	0	0	0·02	0
0·72	0	0	0	0	0



Curve 3. The Reduction of Acidity in Soils treated with Calcium Oxide and stored in a moist condition.

The reduction in acidity with increasing applications of lime is, in the case of the Woburn soil, gradual and not strictly proportional to the amounts applied, but this is not surprising in view of the fact that the soil during the period of storage only contained initially about 18 per cent. water and that some of the lime applied could not be expected, on account of the relatively large size of the particles, to come into action with rapidity. As against this we have the case of the Craibstone soil where the reduction in acidity is directly equivalent to the amount of lime supplied in the two lower dressings. Beyond this there occurs a perceptible "lag" in the curve.

The relation between soil acidity and responsiveness to the application of calcium carbonate is well demonstrated by the increased rate of

ammonia and nitrate production (as an indication of increased bacterial activity) and the amount of plant growth in these soils.

Full details have already been given elsewhere (37), but the following table will serve to bring out this relation more clearly.

TABLE VII. *The Relation between Soil Acidity, the Production of Ammonia and Nitrates, and Plant Growth in certain Soils.*

Soil	Lime req. (as per cent. $CaCO_3$)	Increase in ammonia and nitrates (parts per mill. dry soil)		Plant growth (production of dry matter in 4 crops)	
		Unt. soil	Soil + $CaCO_3$	Unt. soil	Soil + $CaCO_3$
Rothamsted	nil	7	6	100	105
Chelsea	nil	49	44	100	99
Millbrook	0·02	7	12	100	97
Woburn*	0·26	40	84	100†	740†
Craibstone	0·43	26	47	100	144

* The authors desire to express their indebtedness to Dr J. A. Voelcker, Director of the Station, who very kindly placed this and other samples of soil at their disposal.

† From other experiments, first crop only.

The two soils, Rothamsted and Chelsea, contained initially an abundance of carbonate (2·6 and 0·8 per cent. respectively) and the further addition of chalk gives returns varying only slightly from those of the control soils; Millbrook soil, which is almost neutral, gives a slight increase in nitrates and a depression in plant growth. The two acid soils, Woburn and Craibstone, respond readily to chalk applications as indicated by increased nitrate production and plant growth in the first four crops after treatment.

In another experiment the addition of calcium carbonate to a soil showing a lime requirement of 0·117 per cent. resulted in an increase of the following barley crop of 30 per cent.

Having shown that the method suggested provides a good indication of preceding treatment of the soil in the laboratory, some further determinations with field soils may be of interest. The samples used in these experiments were obtained from the permanent wheat and barley plots of the Woburn Experimental Station, already well known on account of their pronounced acid character as a result of continued applications of sulphate of ammonia. Up to 1897 the crops on some of these plots had already begun to fail, while a preliminary application of

lime sufficed to bring crop production to its normal level. Since 1898 various other dressings with lime have been made, in each case with marked benefit (38), and the effect of these dressings is reflected in the reaction of the samples at the present date. For purposes of comparison we have included in the following table the carbonate content of the soils and the yields of barley for the 1913 crop. Full details of the manuring, etc., are to be found in the official reports of the Woburn Station.

TABLE VIII. *The Lime Requirements of Certain Field Soils.*

(Permanent Barley Plots, Woburn Experimental Station.)

Soil treatment	Lime (CaO) applied equiv. to $CaCO_3$ per acre	$CaCO_3$ present 1914	$CaCO_3$ required 1914	Crop yield 1913 bushels
2*a* sulphate of ammonia (25 lb. ammonia)	—	0·003 %	0·260 %	—
2*aa* as 2*a*, with 5 cwt lime 1905, 1909, 1910 and 1912	1·8	0·008	0·140	11·9
2*b* as 2*a*, with 2 tons lime 1897, reptd. 1912	7·2	0·196	—	34·3
2*bb* as 2*b*, with 2 tons lime reptd. 1905	7·2	0·026	0·043	16·3
5*a* mineral manures and sulph. ammonia	—	—	0·183	—
5*b* as 5*a*, with 2 tons lime 1897 reptd. 1912	7·2	0·111	—	31·5
5*aa* as 5*a*, with 1 ton lime 1905	1·8	0·006	0·140	13·1
8*a* and 8*b*, mineral manures and (in alternate years) sulphate of ammonia (50 lb. ammonia)	—	—	0·190	—
8*aa* and 8*bb* as 8*a* and 8*b*, with 2 tons lime, 1897 reptd. 1912	7·2	0·111	—	31·6
1 and 7 unmanured	—	0·006	0·123	6·7

Without necessarily indicating that the controlling factor in crop production of these plots is one of physiological resistance to soil acidity, there is still a very close agreement between yields and soil reaction. In all cases where the soil is neutral in reaction, high returns are obtained; where the requirement is more than 0·18 per cent. the crop shows almost if not complete failure. In addition to an improvement in conditions for plant growth, bacterial processes are speeded up so that, while in our experiments the amount of ammonia and nitrate formed in the untreated soil within the first fifty days was only 7 parts per million, that in soil with only 0·2 per cent. calcium oxide reached

26 parts. Somewhat similar data were obtained with the soils from the permanent wheat plots, although in this case the crop was more resistant to acid conditions, and persisted until the soil showed an absorption of over 0·22 per cent.

The Relation between Lime Requirements and Calcium Carbonate Content.

The relation between the lime requirements of a soil and the amount of calcium carbonate contained therein is of importance in certain circumstances. In the first place, the mass of data now being collected under numerous soil survey schemes is open to misinterpretation on account of the view commonly held that normal soils must contain a certain (often purely arbitrary) content of carbonate. Secondly, and related to the first consideration, field experiments which are initiated on the basis of a low carbonate content of the soil are sometimes liable to give only negative results. This has already occurred from time to time.

Hall and Russell (39) and later Gregoire and his colleagues record a considerable number of soils neutral in character and yet possessing little or no carbonate. We have also met with many of this type, chiefly in our case, light sandy soils. Conversely, many acid soils are encountered where a certain amount of carbonate is present, but this appears to be largely due to localisation of the carbonate (posssibly past applications of lime) in the field. Some examples of each type may be found in the following table, and the authors wish to emphasize the fact that acidity—and not merely carbonate—determinations should be made in estimating the needs of any particular soil.

	Rothamsted	Chelsea	Devon	Millbrook	Geescroft	Metchley	Harper Adams	Woburn	Craibstone	Leeds II
$CaCO_3$ present %	2·660	0·890	0·003	0·035	0·005	0·097	0·005	0·003	nil	nil
$CaCO_3$ req. %	nil	nil	0·015	0·032	0·100	0·117	0·135	0·260	0·430	0·470

The Relative Values of Calcium Oxide and Carbonate for Soil Neutralisation.

Attention has already been called to the essential difference in action between oxide and carbonate when added to neutral soils, the former exercising a specific effect. In the case of soils lacking in lime this difference was not distinctly evident, but on account of having in most cases applied a large excess of carbonate it was difficult to draw fair comparisons with these soils. It was evident however that in the case of the Woburn and Craibstone soils, the net effect of the two forms of lime was approximately the same and it was decided to carry out pot experiments in order to bring out this similarity if possible.

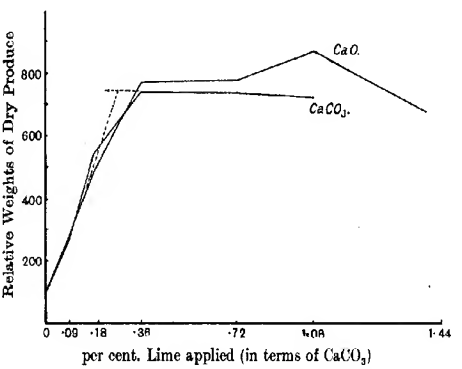
Small glazed earthenware pots were filled with equal quantities (3 kilos) of Woburn acid soil and various dressings of calcium oxide and carbonate (in equivalent quantities) were made, in each case in duplicate. The soils were watered after treatment and in order that crop growth should not be limited by lack of potash and phosphates, an addition of 0·5 grm. of potassium phosphate (neutralised in solution to litmus) was made to each pot of soil. After being allowed to stand in a moist condition for about seven weeks, the soils were stirred up, barley seed was sown and the plants allowed to grow to maturity, that is, about fourteen weeks from the date of sowing. From germination onwards little difference was perceptible between the effect of the two forms of lime and this was also borne out by the actual weights of the plants when cut. These dry weights and relative weights are included in Table IX and are also plotted in Curve 4.

TABLE IX. *The Relative Values of Oxide and Carbonate when applied to Woburn acid soil.*

Crop, Barley.

	Oxide		Carbonate	
	Dry weight	Relative	Dry weight	Relative
Soil untreated	1·52 grm.	100	1·52 grm.	100
Soil + 0·05 % CaO or 0·09 % $CaCO_3$	4·25	279	4·08	268
Soil + 0·10 % „ or 0·18 % „	7·25	477	8·27	544
Soil + 0·20 % „ or 0·36 % „	11·67	768	11·27	741
Soil + 0·40 % „ or 0·72 % „	11·78	775	11·21	737
Soil + 0·60 % „ or 1·08 % „	13·17	866	10·69	703
Soil + 0·80 % „	10·26	675	—	—

These results possess several points of interest. In the first place the returns in plant growth are almost directly proportional to the lighter dressings of carbonate supplied. Little difference is evident between the yields resulting from the application of the lower equivalent doses of oxide and carbonate until the requirements of the soil are satisfied, i.e. the neutral point[1]—the slightly lower value with 0·10 per cent. of oxide being due to an exceptional variation in the duplicates, the other value being 8·20 grms. as against 8·27 grms. with carbonate.



Curve 4. The Relative Effect of Calcium Oxide and Carbonate on Plant Growth in an acid Soil (Woburn). Crop, Barley.

Secondly, the specific action of the oxide only becomes evident with 0·6 per cent. while the next higher dressing with this compound produces a relative depression. These two high dressings were, in fact, made on the results of the titration method described in the first part of

[1] This is of considerable practical importance; the view is often expressed, and great stress has been laid upon it in the United States, that carbonate of lime (chiefly ground limestone) is the only form of lime that can be safely used in practice, on account of the tendency of caustic lime to "burn up" the soil. The results obtained by us in laboratory and pot culture experiments appear to denote that until the reaction of the soil is fully corrected, various decomposition changes proceed with similar rapidity with either form of lime. It is only when excess quantities of oxide are employed—and this appears to have been the case in several American and English experiments—that a sudden liberation of soluble nitrogen compounds occurs in the soil, and local circumstances then determine whether this plant food is assimilated by the first crop or is subject to leaching during the following rainy season. If the latter takes place a heavily limed plot is liable to become impoverished to a greater extent than less heavily or even unlimed soils, and by decreased fertility in subsequent years tends to give support to the prevalent view of the "burning" action of the caustic form.

this paper, where it is shown that 0·6 per cent. constitutes the critical dressing capable of producing the maximum first crop, while 0·8 per cent. is too high for this soil.

Finally, the results are of interest in connection with the question of acidity as indicated by the suggested bicarbonate method. Although maximum growth of the crop is produced by an application of 0·36 per cent., the preceding deviation in the curve might fairly be taken as an indication that this amount is actually in excess of the requirements. If therefore the two linear portions of the curve—the initial and the final—be continued as is shown by the dotted lines, it will be found that the point of intersection occurs above the point already indicated as being the acidity by the bicarbonate method, namely, equal to 0·260 per cent. calcium carbonate. Without wishing to imply that with all soils the crop growth is directly in inverse ratio to the acidity of the soil, a close agreement appears to occur in this case between the lime requirement as shown by the bicarbonate method and the physiological gauge set by the plant.

The Relation between Soil Reaction and Bacterial Activity.

In discussing the necessity of maintaining an adequate supply of base in cultivated soils, it is generally recognised that the presence of carbonate is requisite for the maximum activity of nitrifying and nitrogen-fixing organisms, but little attention is paid to the preceding putrefactive or ammonification process. The work of Russell (40) and others shows that the amount of nitrate formed in field soils is strictly dependent on this preceding change, in fact, the ammonia formed is nitrified almost as soon as produced, hence the ammonia content of normal soils is invariably low.

In our own work with the Craibstone soil (which is free from carbonate) the amount of free ammonia is kept at a low level by continuous nitrification, while even with the Woburn soil the reserves derived from the fertilisers applied are subject to a steady change into nitrates. It appeared of interest, therefore, to ascertain how far a supply of calcium carbonate would affect each of these processes in the Craibstone soil, and two experiments with this end in view were made. It was obviously necessary for the demonstration of increased ammonification that nitrification should be eliminated and this we accomplished by treating the soils with toluene. Equal lots of 600 grms. of the moist soil were filled into bottles and these were divided into three sets. Of these the soil

in set (*a*) was allowed to remain untreated, that in set (*b*) was treated with 10 c.c. of toluene per bottle, while that in set (*c*) received the same quantity of toluene and 2·0 per cent of calcium carbonate. After two days the toluened soils were spread out on sterilised paper in order to allow the antiseptic to evaporate and then returned to the bottles. The analytical data are given in Table X.

TABLE X. *The Effect of Calcium Carbonate on Ammonification.*

Treatment	Ammonia and nitrate (expressed as pts. N per million of dry soil) produced after					
	32 days			70 days		
	Ammonia	Nitrate	Total	Ammonia	Nitrate	Total
Untreated soil	2	39	41	—	44	44
Soil + toluene	17	35	52	27	31	58
Soil + toluene + $CaCO_3$	26	29	55	47	30	77

With the cessation of nitrate production the effect of calcium carbonate on ammonia formation becomes evident after 32 days, but this becomes still more pronounced by the end of the second period of the experiment, the gains during this time with the untreated soil, toluened soil and toluened soil with carbonate being 3, 6, and 22 parts of nitrogen respectively. It is evident therefore that ammonification is subject to retardation in this soil.

TABLE XI. *The Effect of Calcium Carbonate on Nitrification.*

Treatment	Nitrate (expressed as pts. N per million of soil) produced after	
	20 days	57 days
Untreated soil	43	44
Soil + 0·10 % $(NH_4)_2SO_4$	77	131
Soil + 0·10 % $(NH_4)_2SO_4$ + $CaCO_3$	199	205

The second experiment consisted in the determination of the nitrates produced on the addition of ammonium sulphate to the soil, the various sets comprising (*a*) soil alone, (*b*) soil with 0·10 per cent. ammonium salt, and (*c*) soil with a similar amount of ammonium salt and 2·0 per cent.

calcium carbonate. The results, contained in Table XI, show the very great rapidity with which this change can proceed in the presence of carbonate, the change having reached completion within the first 20 days. The amount of nitrification taking place in the soil without lime was also quite appreciable in spite of the lack of free carbonate, although calcium may be liberated from the mineral or the organic soil constituents.

The main effect of an application of carbonate to this soil consists apparently in speeding-up both of ammonification and nitrification, but on account of the reasons stated above, the former action is probably the more important.

The Relation between Soil Acidity and Natural Vegetation.

While the conventional method of soil analysis with reference to lime generally consists in an estimation of carbonates, there are many instances in which this procedure fails to give an index to differences often apparent in the field. This applies to the distribution of plants on many of the heaths and commons throughout the country and also to a certain extent to the vegetation prevailing on much old grass land. As an instance, the case of the Harpenden Common may be cited, where carbonate determinations in soil samples from parts of the Common have failed to provide data that would help to grade the soils as to their relations to plant growth. All these soils react acid to litmus, but examination shows that from place to place, vegetations occur in which white clover, fescues, gorse, sorrel, bracken, etc., predominate.

A large number of these soils have now been examined by means of the bicarbonate method described above and we have been enabled to classify them in terms of acidity and vegetation. A few of these results are given below to illustrate some of the differences encountered. Photographs of typical turfs are shown in Plate I.

TABLE XII. *Lime Requirement as related to Vegetation.*

Average lime requirement of soil	Dominant flora
Approx. 0·22 % $CaCO_3$	Wild white clover (*Trifolium repens*)
„ 0·26 % „	Fescues (*F. ovina* and *rubra*)
„ 0·31 % „	Mixed. Yarrow, woodrush and moss
„ 0·39 % „	Gorse
„ 0·43 % „	Yorkshire fog
„ 0·53 „	Sorrel

1

2

3

4

5

This gradation appears to apply quite generally to the soils on this formation (clay with flints, overlying chalk), but need not necessarily mean that on other and different soils the above plants are associated with the acidities[1] already given. There seems to be little doubt from general observation that clovers are least and sorrel most acid-resistant (neglecting the terms calciphagous and calcifugous), but the extent to which these various plants persist on any given soil is probably determined partly by reaction and the amount of water contained by that soil at any period. In this connection organic matter by helping to retain soil water might conceivably play a part in regulating these inter-relations, which go to form a subject of agricultural importance and of ecological interest.

SUMMARY.

The experimental results described in the two parts of the paper may be summarised thus:

PART I. *Lime Requirements for Sterilisation Purposes.*

(1) The capacity to produce partial sterilisation effects is a property belonging to calcium oxide (caustic lime), but not to calcium carbonate (chalk, limestone, marl, etc.).

(2) The amount of lime necessary to produce specific effects in different soils has been found to vary greatly and it is not possible to make any general recommendations.

(3) The method proposed for indicating the critical amount required is based on the determination of the amount necessary for the production of an alkaline reaction of the soil water.

(4) The amounts thus indicated agree very closely with those required for the production of typical partial sterilisation effects in the soil itself, e.g., the inhibition of protozoa and of nitrifying organisms.

(5) By correlation of the results obtained by the proposed method with those of pot experiments, it is evident that the amount indicated coincides with that required for (*a*) the maximum production of dry matter in the first crop following treatment—heavier applications tending to be injurious, and (*b*) the maximum production of dry matter in the first *four* crops. Applications of lime double or treble the amount indicated by the method, although causing an increase in the

[1] The types of "soil acidity" or lime requirement vary greatly and require further study in their relation to plant growth.

ammonia and nitrates produced, do not give corresponding increases in crop.

(6) Certain physical changes also occur about the partial sterilisation point.

PART II. *Lime Requirements for Neutralisation Purposes.*

(1) The method described for the determination of the lime requirements of the soil is based on the absorptive capacity of the soil for calcium carbonate (present in solution as bicarbonate), which is the chief form coming into operation in the field.

(2) The comparative tests of various soils to which quantities of lime had been added previously, showed proportionate diminution of the lime requirements.

(3) The method possesses the advantage over several others suggested in that it indicates no absorption in the case of neutral soils.

(4) Soils showing a positive lime requirement according to this method have been found to respond distinctly to the application of carbonate (*a*) by increased ammonia and nitrate production in laboratory experiments, and (*b*) by greater plant growth in pot culture and field work.

(5) The application of lime to field soils is reflected in decreased lime requirements and increased crop production even after a prolonged period (upwards of 17 years).

(6) The values of calcium oxide and carbonate have been shown to be identical provided that the lime requirements (for neutralisation purposes) are not fully satisfied. After the neutral point is reached calcium oxide exercises its specific effect. With an acid Woburn soil the returns in plant growth were proportional to the reduction in acidity.

(7) An application of carbonate to a soil exercises a marked effect in accelerating the process of ammonification, and to a lesser degree nitrification.

(8) The results of an acidity, or lime requirement, test and not those of determinations of free carbonate should be taken into consideration when the needs of any particular soil are concerned.

(9) In the case of soils on the same geological formation a definite relation between soil reaction and natural flora has been traced. The occurrence of certain plants on acid soils appears to be determined by their capacity of resistance to acidity.

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NOTES ON SOME METHODS FOR THE EXAMINATION OF SOIL PROTOZOA.

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(With Plates II and III.)

I. Introduction.

During the last three or four years, the protozoa of the soil have been the object of a considerable degree of interest, and investigations into their occurrence and importance have been made by workers here and elsewhere. The aim of the present paper is to indicate what we know of the life of the protozoa in the soil, and to furnish descriptions of certain methods which have been found useful in work on this subject.

When attention was directed to the protozoan inhabitants of soils, it was quickly found that protozoa in great numbers and variety were easily obtained by inoculating soil into a suitable medium. Setting out from this fact, investigators have frequently been led to describe the forms found in cultures from a soil as the fauna of the soil, thus making the more or less tacit assumption that every form found in cultures from a soil was leading a trophic life in the soil at the moment when the culture was made.

Unfortunately, what may be termed the "cultural fauna" of a soil is of relatively little value in forming an idea of the protozoa actually living in the soil. On the one hand the cultural fauna consists in part of protozoa which were present in the soil only as cysts; on the other, some forms relatively important in the soil, notably the thecamoebae, appear very late, or not at all, in cultures on the ordinary media.

The protozoa in any soil may occur in the active (trophic) state, or enclosed in cysts. We propose to call the former the "active fauna," and the latter the "cyst fauna"; and we would emphasize the necessity of keeping these two classes clearly distinguished.

Under the varying conditions which obtain in a soil there must be continually changing relations between these two faunas, but at any moment only the members of the active fauna of that period can exert any effect on the soil. To guard against possible misunderstanding, it may be well to state that it is very improbable that the line between the active and the cyst fauna of a soil is one between species and species. There is little doubt that under most conditions a species represented in the active fauna will also be represented in the contemporaneous cyst fauna.

Since the cultural method fails to distinguish between the above two categories, and even leaves unsettled the question of whether an active fauna is present at all, recourse has been made to other methods of examination, which are fully described in the next section. By their aid it has been completely established that an active fauna does exist in a variety of soils ranging from the unmanured plot on Broadbalk field at Rothamsted to sewage-farm soil, leaf mould, and soil from a cucumber border. Some of the results obtained by the examination of these soils will be found in section III.

As regards the forms found, it is improbable that many are generically new; most of them seem to have been described by the older workers on protozoa. Of recent years a very large amount of the literature on protozoa, including the more recent textbooks on protozoology, have been devoted almost exclusively to parasitic forms, so that a worker on soil forms must refer back to the excellent papers of the older authors. References to some of these works will be found in the literature list.

Before the effect of protozoa on the soil can be adequately discussed, it is necessary to gather information about the life led by the protozoan fauna. In particular the effect exercised on the active fauna by the water content, the density of the bacterial flora, the temperature, etc., must be investigated.

Now whilst soil temperatures can readily be determined with sufficient accuracy, the evaluation of the other two factors presents considerable, and in part unsolved, difficulties, which arise largely from the heterogeneity of the soil.

Thus the present method for determining water content deals usually with samples taken to a depth of nine inches. It is clear, however, that if in dry weather a crust has been formed on the surface of the soil, the protozoa may be active at a lower level which might still have a relatively high water content, so that the figure obtained

for the water content of the whole soil would give no indication of the actual minimum quantity of water in which protozoa could remain active. This difficulty would be felt even if the soil were a homogeneous mixture; but unfortunately this is far from being the case, and it is certain that in a relatively dry soil the fragments of manure and of decaying plant roots would hold a far larger amount of water than is indicated by an ordinary determination of the water content, so that if, for example, one kilogramme of soil contained 950 grammes of soil particles and 50 grammes of decaying organic matter on which protozoa were flourishing, the figure given by the estimation of the amount of water present in the soil would give no indication as to the actual amount of water in the space where these protozoa were leading an active life.

Another important question is the difference between a coarse grained and a fine grained soil with an equal percentage of water. It would seem quite possible that an active protozoan fauna would be found in the large water spaces in the former at a time when the latter would exhibit no free forms.

Further, when conditions in different soils are to be compared, it is preferable from the biological point of view to express water content as percentage by true volume rather than as percentage by weight.

As regards bacterial counts, all the points which have been urged in connection with the heterogeneous nature of the soil carry here even more weight. In the first place, it is probable that the bacteria are concentrated in groups round decaying organic matter, and it has been found in the examination of fresh films from the soil that the bacteria are present as colonies, and are not scattered singly like currants through a cake. It is obvious that the bacterial count must very largely depend on the degree to which these colonies are broken up during the process of dilution. It is well known, also, that the numbers obtained are dependent upon the medium adopted, and on the conditions of culture.

When the heterogeneity of the soil is taken into consideration, it would seem impossible to hope for an accurate method for the estimation of the active protozoa present in a soil. It is, however, possible that practicable approximate methods may be devised, but before they can be considered satisfactory as a basis for extended experiments, it is very necessary that the range of their probable error should be known.

Up to the present, the only method proposed for the enumeration of the soil fauna is the dilution procedure described by Rahn (11). The work of Cunningham and Löhnis (3) on the thermal death-point of the

active, and of the cyst fauna, has been used by Cunningham (4) as the basis of a method of determining the active fauna. He estimates the total fauna of a soil, and, in a second sample, the cyst fauna; the difference between the results is taken as a measure of the active fauna.

Unfortunately, the results obtained by a dilution method will almost certainly be vitiated by the incompleteness of the cultural fauna. As has already been pointed out, present cultural methods fail to indicate, or indicate very late indeed, an important class of soil protozoa, the thecamoebae. Again, the manipulative errors of the successive dilutions, together with the serious risk that shaking will not result in an even distribution of the protozoa through the suspension, introduce a cumulative series of inaccuracies into a troublesome and complicated method. Finally, in common with any other numerical method, it encounters the weighty difficulty of the heterogeneous nature of the soil.

On the whole, therefore, it seems to us that this type of method will be liable to introduce a specious appearance of accuracy into a subject which bristles with difficulties[1].

A very rough, but still valuable, idea of the relative abundance of active protozoa in soils is given, however, by the richness of the fresh fixed films obtained as described on p. 112. In comparing different types of soil only the most striking differences can be regarded as significant, but in considering the variations in the active fauna of one particular soil under changing conditions of temperature, moisture, etc., it is probable that the index of richness of the films obtained will prove a sound basis for general conclusions, although no hope can be entertained of reaching numerical results by this method.

II. Methods.

It is exceedingly difficult, in an examination of any ordinary soil, to get an adequate idea as to the abundance and nature of the active fauna, and for this reason we have thought it well to describe some of the methods we have found helpful in this work.

By far the simplest method of fixing and staining soil protozoa, whether in cultures or on fresh films from the soil, is by means of coverslip films. We have usually stored the films in small corked tubes of height $1\frac{1}{2}''$ and diameter $1\frac{1}{4}''$, and these tubes have been found very convenient for purposes of fixation and staining.

[1] This criticism does not apply to Cunningham's paper, where it is recognised that precise numbers cannot be given.

If ordinary coverslips are used for this work it is often difficult to decide which side of the coverslip the film is on, particularly if the films have been stored for some time in 70% alcohol. For this reason the coverslips described by one of us in a previous paper ("A note on the protozoa, etc., from sick soils," *Roy. Soc. Proc.*, Vol. 85, 1912, p. 395) will be found very useful. These are oblong coverslips of which one angle has been cut off, and they can be procured from Messrs Frazer, of Edinburgh, Messrs Zeiss, or Messrs Angus. It is obvious that no mistake can arise if it is arranged that the film is always on the lower surface of the coverslip when the long sides point away from the worker and the cut corner separates the right long side from the distant short side.

The methods for the examination of soil protozoa can be divided roughly into three categories, (1) methods for the detection and examination of the active fauna in life, (2) methods for the examination of the active fauna on fresh fixed films of a soil, (3) cultural methods.

(1) *Detection of active fauna in life.* Up to the present we have found no reasonably successful method for the collection and examination of the active fauna of a soil in a living state. Any method which depends upon the addition of water to the soil must admit of very rapid execution, otherwise there is the danger of protective cysts present in the soil opening, and thus giving a false impression as to the constitution of the active fauna. This danger is probably a very real one in the case of the small flagellates, and especially the resting forms of some green algae, in the case of which a few minutes' immersion in water may make the difference between a resting and an active form. Another difficulty seems to be to obtain films adequately rich in comparison with the films got by fixing the fresh soil by the methods described below, and in this respect it is found that methods which give fair results with one type of soil may break down completely with another.

All the methods we have used with any success up to the present depend upon the possibility of collecting and retaining some of the protozoa on a surface film. They all seem uniformly bad, and the only consolation in their use is that the other methods we have tried, including the use of the centrifuge, have up to the present given worse results.

With some rather dry, clay soils, at Rothamsted, fair results were obtained by crumbling a soil into a dish of water, and removing the surface film for the purpose of examination either by floating coverslips on it, or by means of thin wire formed into a circular loop of about $\frac{1}{2}''$ diameter.

In the rather coarse, sandy soils, at Abergavenny, fair results were obtained in the case of small flagellates, thecamoebae, and small amoebae, by allowing a stream of water to flow from the tap on to a quantity of the soil in the dish, until the soil was just covered, and then examining the surface films collected as above.

In the case of rather dry, clayey soils at Rothamsted, fairly large amoebae, with a thick pellicle, were obtained by the bubbling process described below.

A glass tube of internal diameter $1\frac{1}{2}''$ and length about 2′ is provided with a singly-bored rubber cork at the lower end. Through this passes a glass tube drawn out to a jet. Connection is made with some form of airblast, so that a stream of air can be blown through the jet. The tube is clamped upright and a newly made suspension in water of the soil to be examined is poured in until the water level nearly reaches the top. Three hooks (conveniently made of bent strips of "tin") are hung round the rim of the tube in such a way as to furnish a support for the coverslip. The coverslip is placed in position about $\frac{1}{4}''$ above the water level. Air is now blown through the jet so as to produce a stream of fairly small bubbles rising through the suspension and breaking on the lower surface of the coverslip. The water level can be adjusted within small limits by regulating the air-flow.

After about 30 seconds the air-stream is stopped, and the coverslip lifted off and examined under the microscope. It is frequently of advantage to place a thin sheet of agar jelly on the lower side of the slip before commencing the bubbling, as the protozoa adhere more readily to this substance than to the glass. If this be done, the coverslip is placed for examination, agar side up, on a slide, and another slip is dropped on to the agar surface.

By this method there were obtained from a Rothamsted soil certain amoebae whose presence in the active fauna the other methods had failed to reveal.

Very fair stained preparations of any of the animals obtained by one of the above methods can be made by the ordinary processes in use in the zoological laboratories for making preparations under a coverslip. The easiest method is probably to fix by running a drop of Fleming's solution under the coverslip for a few seconds, then washing through with water, followed by picro-carmine five to ten minutes (this renders the process of staining after the Fleming fixation much easier), washing through again with water, staining with alum carmine for half an hour, washing through again with water, then alcohol up to

absolute, followed by terpeneol and balsam. Terpeneol will be found very convenient for this purpose as it clears from a much lower percentage of alcohol than oil of cloves or oil of cedar.

(2) *Examination of active fauna in fresh fixed films.* In the preparation of fresh films from soil to which a fixative has been added we once again depend upon the surface films. For some obscure reason not yet understood, if certain fixatives are added to a quantity of soil a surface film is formed which contains an unknown but probably variable proportion of the active fauna of the soil, cysts, diatoms, moulds, algae, and bacteria. In the production of this result, it is certain that the contained air in the soils exercises a favourable influence in bringing the animals to the surface film, and really good results cannot be expected by this method from a soil which is absolutely water logged. Of the fixatives we have tried up to the present, picric alcohol (*i.e.*, 50 % saturated solution picric acid in water, plus 50 % rectified spirit), and corrosive alcohol (*i.e.*, 50 % saturated solution corrosive sublimate in water, plus 50 % rectified spirit) have given us the best results.

The best method appears to be to place the soil in a porcelain dish, and pour enough of the fixative through a funnel to the bottom of the soil layer until the soil is just covered. The film so obtained can be taken off on coverslips floated on the surface of the liquid.

Of these two fixatives picric alcohol appears to give richer and more abundant films, particularly as regards small organisms, in sandy soils, whereas corrosive alcohol appears to work better on clay soils, and is more efficient in collecting thick-pellicled amoebae.

The efficiency of the film formation is frequently increased by slightly shaking the dish immediately after the addition of the fixative. The following is a good method for staining and mounting the film so obtained.

Picric Films Corrosive—2 minutes	Corrosive Films
70 % alcohol plus a few drops of I_2 in KI	5 minutes
Distilled water	5 minutes
Haemalum	5 minutes
Tap water	Till blue
70 % alcohol	5 minutes
Eosin in absolute alcohol	3 minutes
Absolute alcohol I	1 minute
Absolute alcohol II	1 minute
Xylol I	2 minutes
Xylol II	1 minute

The over-staining in eosin will be found of great assistance in searching rather poor films for active forms, especially in the case of flagellates.

These methods have been found to give very fair results as regards small flagellates, small amoebae, and thecamoebae. Up to the present we have only very rarely found large flagellates and ciliates, but to this question we return in a later part of the paper.

(3) *Cultural Methods.* It would we feel be premature at present to attempt a formal list of the culture media on which soil protozoa flourish. In all cases of cultures of soil protozoa, so far as we are aware, as Vahlkampf clearly insisted in his paper on the biology, etc., of *Amoeba limax*, the protozoa feed upon the bacteria of the culture, and hence almost any culture media on which soil bacteria flourish will probably support a large number of protozoa.

Therefore in those cases in which the expression "pure animal culture" is used we only wish to indicate that the culture contained only one form of protozoon, though of course it contained large numbers of bacteria. It may of course be possible in the future to obtain cultures of some saprozoic protozoa free from bacteria, and in certain cases we have found indications that certain amoebae show a distinct preference for certain culture media, though here, again, this effect may be a secondary one due to the encouragement of a certain type of bacteria.

Up to the present we have mainly used solid media for our cultures, as we find that they are far more convenient for isolating any given form. We used two types of culture media, one an ordinary agar made up of 1000 c.c. meat extract and 15 grm. of agar; but we have found a culture medium of Friedberger and Reiter described in Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen*, vol. I, gives very good results for most soil protozoa; it consists of a horse-dung agar made up of three lumps of horse dung and 500 c.c. of water, this mixture is boiled for one and a half hours, then filtered through cloth, and finally about 8 grm. of agar is added. In many cases where it is used to get a very strong growth of protozoa it is advisable to add a small amount of water or dilute albumen to the culture plates to about a depth of 2 mm. This addition of water seems to obviate the vacuolated appearance which some workers have noted as characteristic of culture amoebae on plates.

The stock cultures are made up by adding a little soil directly to the plates. If these stock cultures are examined from time to time it will be found that in any given culture there is a more or less definite

succession of animal forms. By selecting the time and method of culture it will probably be found possible to get pure animal cultures of any of these forms.

The question how far the dominant active forms in a soil are represented in the cultures depends largely, firstly, on the condition of the soil, and, secondly, on the condition of the cultures. We return to this question below, but it may be pointed out here that in the case of most soils the conditions on the cultures mentioned above seem rather rich for some members of the active fauna, with the result that these forms appear very late in the cultures. A certain check can be obtained on these results by means of cultures in which a small amount of water is added to the soil.

III. Some Results.

So far the soils which have been examined by the methods described above are relatively few in number, but of varied types.

In three cases, where the soil was taken respectively from a cucumber border, from a fertile garden plot, and from the site of an old manure heap, the soils were probably far richer in farmyard manure than even the most richly manured fields; and correlated with this there was a higher capacity for holding water. As would be expected, all the indications were that these soils supported a far denser protozoan fauna than was found in the poorer soils examined.

In the cucumber border, the dominant protozoa were amoebae—one of the limax type (*Vahlkampfia soli* n. sp.) and one of the lamellipodian type (*A. cucumis* n. sp.). Thecamoebae, notably a species of Euglypha and a Trinema, could be detected in live films, though they were fairly rare on the fixed films, and were probably the next most numerous protozoa. Flagellates and ciliates were present only in small numbers.

The garden soil, and the soil taken from the site of an old manure heap (both at Abergavenny), contained many amoebae, but a great preponderance of thecamoeban forms. The similarity between their fauna is probably not accidental; it is very likely that the dominance of the thecamoebae in the garden soil was a persistence of the dominance of these protozoa in the manure heap with which the garden had been enriched.

In culture these thecamoebae did not appear in considerable numbers until two or three weeks at least after the culture had been started.

From a consideration of cultural results alone, it would have been imagined that flagellates, both large and small, and amoebae had been the dominant forms.

In a not very rich soil from a cauliflower seedling bed the picric acid method gave a considerable variety of protozoa, no one form of which appeared to have become predominant. It was fairly clear that the density of the fauna was relatively low. It is interesting to observe that this rather poor soil contained many more species than *e.g.* the soil from the cucumber border, though the latter had many more individuals. This suggests an interesting analogy with results obtained on the grass plots at Rothamsted, where the untreated (poor) plot gives a large number of species, whereas on plots which have received a large quantity of manure for many years the number of species is considerably curtailed. A similar phenomenon is shown in rich infusions, in which as a rule at any given moment one or other protozoon has got the upper hand, whilst in ordinary fresh-water pools the fauna is far richer in number of species, but far poorer in number of individuals.

The three Rothamsted field soils (Broadbalk dunged plot, Broadbalk unmanured plot, and a fallow plot on Agdell) also contained protozoa very sparsely, small amoebae being the most numerous, though thecamoebae were also represented. Flagellates were very rare, and ciliates were not found at all in the active state.

In culture, amoebae of the two types found in the cucumber border were prominent, together with a great variety of flagellates and many ciliates. The amoebae on the fresh films seem to be of a type different from either the limax or the lamellipodian amoebae.

Rather large amoebae of two sorts, both with a thick pellicle, were obtained from the dunged plot on Broadbalk (14 tons farmyard manure per acre each year since 1843) by the bubbling method. It is possible that these were more resistant to a comparative degree of drought than the more delicate types which flourished in the wet cucumber soil and came on strongly in cultures from the field soil.

By far the most abundant results were obtained with samples of these soils collected in November, 1913, when the moisture content of the dunged plot was given by the usual method as 22 %. In the dry summer of 1914 when the moisture on this plot varied usually between 13 % and 10 %, very poor results were given by all methods of investigating the active fauna. There is a distinct probability that here the water content is a limiting factor in determining the density of the active fauna.

In the case of the Abergavenny garden soil no clear correlation of this kind was observed; observations were, however, only made in the summer (June) before and after rain.

To get an idea of the fauna of a soil very rich in humus, a deposit of black leaf-mould in a wood near Abergavenny was sampled. Here thecamoebae were again very numerous, amoebae were slightly less numerous, and small flagellates and some ciliates were easily detected. As a further example of a soil rich in organic matter, samples were taken from a sewage bed at Abergavenny. Sewage had been led on to this, and allowed to percolate through. When the samples were taken the bed had dried sufficiently to allow of the deposit being scraped up into heaps ready for removal. Enormous numbers of phytoflagellates (forming a green film on the surface) were present, and thecamoebae and amoebae were very plentiful. Ciliates were not uncommon, and the smaller flagellates were fairly well represented.

As far as these results go, it appears that the numerically most important types of soil protozoa are thecamoebae and amoebae. Flagellates and ciliates are relatively rare. Of the flagellates found, it is very noticeable that the larger forms, such as Bodo and Copromonas and their allies, appear so far to be of very little importance in the active fauna. The most successful soil flagellates are small monads. This is a result which is not revealed by cultural methods, when the larger flagellates assume a much more prominent position. Sherman (14), using a dilution method, found small flagellates to be the most abundant protozoa in the soils with which he worked[1]. Though our observations have not, so far, supported his, we cast no doubt on the substantial accuracy of his results.

The results of examination of the Broadbalk dunged plot in winter and in summer suggest that normal variations in water content may have a considerable effect on the active fauna of the soil, but in the present stage of our investigations we feel it would be premature to lay too much stress on this point.

[1] Cunningham (4) arrives at a similar result.

IV. CONCLUSIONS.

It seems probable from the work that we have done up to the present that there are always some free living protozoa present in a trophic state in even relatively dry, poor soils.

In manuring on ordinary soil with farmyard manure, a large number of protozoa are introduced into that soil, and if the conditions of culture are such as to necessitate a high water and a high manurial content, the protozoa may well get the upper hand to such an extent as to produce a well-marked deleterious effect on the crop, resulting in the condition known as soil sickness (*e.g.*, in cucumber beds, sewage farms).

The nature of the protozoan fauna seems to vary to a certain extent with the soil under examination. It is probable that this is largely due to actual difference in the fauna of different soils, but it may be partially due to another factor. As is well known, if some soil is added to a hay infusion or other suitable culture medium, the fauna shows a tendency to run in cycles (*e.g.*, at first the dominant forms would be found to be small flagellates; these are usually followed by larger flagellates and amoebae, and these are succeeded by ciliates). It is possible that such cycles may occur in the soil, and it is possible therefore that two soils with a similar water content may show quite different active fauna, depending on the point of the animal cycle at which that soil had arrived. The dominant protozoa found in a trophic state in a soil may be the dominant form found in the cultures, as was probably the case in some sick cucumber soils; but it of course depends on the suitability of the medium, and the culture method adopted. It is probable that the richer the soil and the higher the water content at the time of examination, the greater the probability of the dominant culture form being the dominant trophic form in the fresh soil. A possible exception to this rule is furnished by the thecamoebae, which usually only appear late under present cultural conditions.

It will be seen that up to the present the dominant active fauna of the soil, as shown by the fresh films, consists mostly of amoebae, thecamoebae and small flagellates.

In this connection there is one point which requires further investigation, and that is the frequent prevalence of relatively large flagellates in soil cultures (*e.g.*, Prowazekia and Copromonas), whereas in fresh films the only flagellates found are very small monads. It may perhaps be found that the Prowazekia are present in the trophic state only in

groups on the decaying organic matter in the soil, possibly only for short periods, and that the encysted forms present in the soil are favoured by the condition of the culture at the expense of the smaller flagellate forms, or it is possible that these large flagellates are contented with a very short trophic life in the soil at a time when the water content is high and there are large quantities of decaying material in the soil.

Under these conditions it is not unlikely that the ciliates so frequently found in soil cultures lead a trophic life in the soil.

There is another factor which must be reckoned with in this connection, and that is the possibility that the present methods for the examination of fresh soil films do not give a fair account in regard to these large flagellates, which may be caught up by their flagella amongst the soil particles.

None of these possibilities is mutually exclusive, and it seems from recent work on cultures of soil to which water alone has been added that the last explanation is not very probable.

In conclusion, it seems to us that there are three categories under which the protozoan population of any soil at a given moment should be studied, (1) the active fauna, (2) the resting fauna (in cysts), and (3) the cultural fauna. In the immediate future better methods must be devised for the detection of the active fauna, a complete study is needed of the possible seasonal variations which might result in a transfer of certain forms from the resting fauna to the active fauna, and a more careful study must be made of cultural conditions, so that it may be possible to cultivate at once any desired member of the active fauna of a soil.

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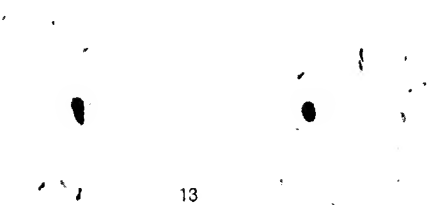

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DESCRIPTION OF PLATES.

PLATE II.

FIG.
1. *Euglypha* sp. from fresh fixed film (see p. 112) of cucumber bed. A thecamoeba.
2. *Chiloden* sp. from fresh fixed film of cucumber bed. A ciliate.
3. Flagellate from fresh fixed film of cucumber bed.
4. Dividing *Vahlkampfia soli* from fresh fixed film of cucumber bed. A limax amoeba.
5. *Euglypha* sp. from fresh fixed film of cucumber bed. A thecamoeba.
6. *Chlamydophrys* sp. from fresh fixed film of cucumber seedling bed. A thecamoeba.
7. *Amoeba gobanniensis* from fresh fixed film of cucumber seedling bed. A lamellipodian type of amoeba.
8. *Amoeba* sp. Do.
9. *Amoeba* sp. Do.

PLATE III.

10. *Vahlkampfia soli* from fresh fixed film of cucumber bed. A limax amoeba.
11. *Vahlkampfia soli* stage in division.
12. *Amoeba cucumis* from young culture. A lamellipodian amoeba.
13. *Amoeba cucumis* late stage in division.
14. *Bodo caudatus* from a culture. A flagellate.
15. *Bodo caudatus* stage in multiple division.

Note. These illustrations are designed to assist bacteriologists and others who are interested in soil protozoology to refer the species they will encounter to the general type. It is hoped in particular that the organisms vaguely referred to as "Amoebae" may be more definitely distinguished at least into Thecamoeba and Amoeba. The limax and the lamellipodian type of amoebae will almost certainly be among the most successful amoebae found in cultures, and it would be of interest to distinguish them from one another and from other less defined types. The sizes of the protozoa shown varied from 15 to 50 μ; but the figures were not drawn to the same magnification.

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SOME CONSIDERATIONS AFFECTING THE GROWING OF LINSEED AS A FARM CROP IN ENGLAND.

I. VARIATIONS IN THE OIL CONTENT.

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(With 2 Text-figures.)

Owing to the rapid rise in the price of linseed and linseed products during recent years, the question has arisen as to whether the farmer can grow linseed, for home consumption, at a smaller cost than he can buy it for under existing conditions. This question involves considerations as to which of the several kinds of linseed would be the most profitable to grow, and it becomes necessary to consider not only the agricultural requirements of the crop[1], but also the fact that the value of this crop is largely, if not entirely, determined by the quantity of oil produced per acre.

Numerous experiments on quite a small scale have been carried out at different times in different parts of the country and, notwithstanding much discordancy, the general indication seems to be in support of the opinion that given the most suitable variety and an average season, linseed growing in this country is a profitable undertaking.

In some parts of the world flax is grown for seed only—*i.e.* as *linseed*[2] —while in other parts it is grown for fibre (seed being a secondary consideration)—*i.e.* as a *line*[3] crop, and the belief has gained general credence, that high quality fibre alone could be obtained combined with inferior seed—inferior, that is, from a feeding point of view. The work

[1] "Notes on Linseed. I. Linseed as a Farm Crop," *Journ. South Eastern Agric. College*, Wye, 1914.

[2] *Vide* "The Growing of Linseed for Feeding Purposes," *Journ. Bd. of Agric.* 1913, xx. pp. 377–385.

[3] *Vide* J. V. Eyre, *Science Progress*, April 1913, pp. 596–628, Supplement to *Journ. Bd. of Agric.* No. 12, Jan. 1914, and *Journ. Roy. Agric. Soc. England*, 1913, **74**, pp. 127–141.

of Ivanoff[1] however goes to prove that this view is erroneous and that there is little difference in oil content between the seed from the fibre crop, *i.e. flax seed*, and that from the linseed crop; and this has been confirmed by the present writers.

Our results indicate that the only difference between the seed from the fibre crop and that from the linseed crop is one of yield, there being little difference in oil content between the two kinds. This is important because it indicates that by harvesting early the best quality fibre may be obtained without materially lessening the oil content of the seed and consequently the value of that commodity. For the only rational way of valuing linseed is on its oil content; oil per acre from the farmer's point of view; oil per ton from the factor's point of view. In consequence of the increased demand for linseed oil it cannot be emphasised too strongly that both farmer and factor must sell or buy linseed on an oil basis and the farmer would do well to realise that this is the only possible basis on which he can sell his crop; that he is, in other words, growing oil rather than seed.

The main object of the present communication is to point out the varieties of linseed best suited to English conditions and giving the largest yield of oil per acre, and also to ascertain the effect of artificial manures on the oil content of the crop produced.

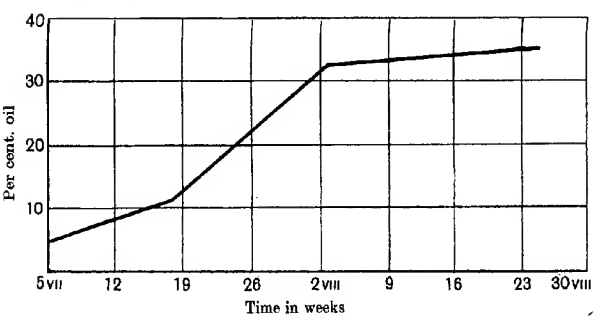
The possibility of harvesting linseed of high oil content from a *line* crop becomes apparent from Ivanoff's work already referred to on the oil-forming processes occurring in oil-bearing seeds. Ivanoff developed the subject from the plant physiologist's point of view and did not concern himself with the agricultural or economic aspect, but his work is nevertheless of considerable interest agriculturally. He estimated the oil present in the linseed at different periods after flowering and his results for one series of determinations with a crop grown in 1910 are shown in the accompanying table and graph.

An examination of these results shows that for the first two weeks after flowering the seed material contains very little oil. After this comes another period of two weeks during which the formation of oil takes place with great rapidity. During the last two or three weeks the increase in oil content was found to be extremely small, amounting to only about 2·5 % increase in three weeks, whereas the increase during the previous two weeks amounted to more than 21 %. The optimum oil formation, therefore, occurs exactly in the middle of the period between flowering and final ripening, and the results seem to indicate

[1] *Beihefte zum Bot. Centrb.* Band XXVIII. Heft 1, pp. 159–191 (Jan. 1912).

that, from this point of view, little is gained by allowing the seed to ripen before harvesting the crop. In view of its agricultural interest an attempt was made by the present authors to repeat this work. As no mention is made by Ivanoff as to the means adopted to secure seed of uniform ripeness at the dates of gathering and as difficulty was

	I	II	III	IV
Harvested	1st week after flowering July 5th	July 18th	August 3rd	August 25th
Percentage oil	4·37	11·00	32·50	35·04



experienced in doing this a different procedure was adopted. Instead of gathering the seed at different dates after flowering, the seed collected at harvest was separated into four groups of increasing degrees of ripeness, viz.:

A. The seed quite green;
B. The seed just beginning to turn brown;
C. The seed wholly brown, but not loose in capsule;
D. The seed fully ripe, *i.e.* quite loose in capsule.

Determinations of the oil content of these various groups gave the following results:

A.	19·86 %;	22·23 %;		mean = 21·045 %;
B.	25·30 %;	29·17 %;	35·77 %;	mean = 30·08 %;
C.	37·41 %;	38·94 %;		mean = 38·03 %;
D.	40·84 %;	40·93 %;		mean = 40·88 %.

Notwithstanding the fact that, in all cases, the analyses were made immediately after gathering the seed, as had been anticipated, it was

not found possible to obtain concordant results with the less ripe samples although such was not the case with the fully ripe seed. Presumably this is due to the rapidity with which oil is accumulated in the seed during the earlier stages of ripening—during the period represented by the more rapidly rising portion of Ivanoff's graph. During this period of development a slight difference in maturity would have a relatively large effect on the oil content and this is indicated by the different results obtained with the three samples of seed in group B. During the later stages of development, in which the oil-forming processes slow up, more concordant results were obtained, as exemplified by group C, while in the final stages oil formation has almost ceased and consequently the oil content remains practically constant.

It is evident that, taken generally, these results are quite in harmony with those of Ivanoff.

Further evidence on this point is afforded by the fact that whenever imported *flax* seed is grown in this country as a *linseed* crop the oil content of the seed obtained invariably approximates to that of the parent seed; and this in spite of the fact that in the latter case the seed is allowed to ripen before the crop is harvested, and in the former case the crop is harvested in an unripe condition. Thus a sample of white flowering Dutch seed, grown in Holland as a flax crop, and pulled early, the seeds being allowed to ripen in the stook, contained 35·49 % of oil. This sample, grown at Wye in 1913 as a linseed crop, gave seed containing 35·08 % oil; and that grown at Harper-Adams Agricultural College in Shropshire had 36·71 % of oil.

Comparison of English grown and imported linseed.

It has been maintained by some writers that English grown linseed is inferior to that grown in the better known linseed growing countries. Thus J. A. Voelcker[1] states that although English grown linseed stands very fairly as regards oil content, it is neither superior nor equal to the best grown foreign seed and in support of this contention quotes the following figures which were obtained from various sources:

Black Sea	38·4 %	English grown	36·7 %
Bombay	38·2 „		33·5 „
St Petersburg	35·3 „		32·8 „
Alexandria	35·7 „		
Riga	34·7 „		

Further he stated that he had been unable to find any instance of English grown linseed that was better than the foreign linseed, and

[1] *Journal of Farmers' Club*, 1897, p. 65.

"the average, as one would naturally expect in a climate such as ours, must be considerably below what is produced in India, and in countries where the climate is favourable to the production of oil." As the figures quoted below will indicate, such a view is entirely opposed to our own experience.

In the first place it is necessary to point out that the figures quoted by Voelcker do not constitute a real comparison since he says nothing of the nature or source of the English grown seed: one cannot compare an imported Bombay seed with an English grown Dutch, and there is no indication that this is not done in the examples quoted. And then, too, different samples of the same variety grown in the same region in different seasons show wide differences in oil content—differences that are often far greater than any there may be between English grown and imported seed. This is well illustrated in the following table:

	J. A. Voelcker[1]	A. Voelcker[2]	Leather[3]
Black Sea	38·4 %	30·78 %	—
Riga	34·7	31·19	—
Bombay	38·21	—	40·71%

The only method of obtaining a strict comparison is by comparing samples of the different varieties grown in different parts of the world with the seed produced when the *same samples* are sown in different parts of England. Such a comparison should be carried out during several successive seasons. This is being done: linseed trials are being conducted in accordance with a scheme drawn up by the British Flax and Hemp Growers' Society, Ltd. on the College farm at Wye; at Camblesforth Hall farm in Yorkshire; at the Seale-Hayne Agricultural College in Devonshire; at the Harper-Adams Agricultural College in Shropshire; and at the Holmes Chapel Agricultural College manurial trials are being carried out. Below are given the results for the season 1913, and we hope to continue the work over several years so as to make the comparison a stricter one by eliminating the effect of seasonal variations. It is worthy of note when considering the following observations that the season 1913 was an average one as regards weather and was not specially favourable to the linseed crop, either as regards yield or oil-formation.

[1] *Journal of Farmers' Club*, 1897, p. 65.

[2] "On the characters of pure and mixed Linseed Cakes," *Jour. Roy. Agric. Society*, 1873, pp. 1–51.

[3] "The Comp. of Oil seeds of India," *Mem. of Indian Dept. of Agric.* (Chem. Series), Vol. I. (1907), No. 2, pp. 13–38.

The method employed in estimating the oil content was the ordinary ether extraction method and requires but a brief description. Each sample of seed was carefully examined and only sound seeds were used in the estimation. Damaged or otherwise unsound seeds were rejected as was also adventitious matter such as weed-seeds, chaff, etc. About

TABLE I. *Showing Oil Content of English grown and Imported Linseed.*

Variety of Seed	Imported 1912	English grown 1913	
Pskoff (Imported direct)	37·45 %	36·68 %	(Selby)
Moroccan—Mazagan (London market)	40·60	42·90	(Wye)
		40·13	(Camblesforth)
		39·06	(Seale-Hayne)
		40·86	(Harper-Adams)
Plate (London market)	38·45	42·80	(Wye)
		39·69	(Camblesforth)
		37·72	(Seale-Hayne)
		41·35	(Harper-Adams)
Dutch—white flowering (London market)	35·49	37·69	(Wye)
		35·08	(Camblesforth)
		34·60	(Seale-Hayne)
		36·71	(Harper-Adams)
		34·08	(Holmes-Chapel)
Dutch—white flowering (Imported Gröningen)	38·65	38·30	(Selby, as line crop)
Dutch—Riga child (Imported Rotterdam)	36·13	37·11	(Selby, as line crop)
Steppe—Russian (Liverpool market)	38·90	41·50	(Wye)

Note. The amount of moisture present in the seeds was not determined but an examination of many published figures showed this to be never less than 5·5 % nor above 11 %; while in by far the greater number of cases the amount was between 7 % and 9 %. The variations seem to be largely due to climatic conditions; the hotter the climate the smaller the moisture content.

On the other hand, the constancy in the water content of linseed grown in different parts of the same country under roughly comparable climatic conditions is really remarkable. Leather gives the following for Indian varieties:

Punjab .....7·60 % (Average of 10 samples)
Central Provinces .....6·73 % („ 21 „)
Bombay Presidency .....6·81 % („ 7 „)
Madras Presidency .....6·72 % („ 5 „)

In view of these facts it seemed to us redundant to estimate systematically the moisture present in the seeds we have used because such small variations could not mask (and in some cases would tend rather to increase) the difference in oil content indicated in the table.

5 grms. of the seed was thoroughly ground in a mortar with silver sand which had been extracted with boiling hydrochloric acid and washed free from acid and soluble salts. The finely ground material was transferred to a Soxhlet extraction thimble; anything remaining in the mortar being washed into the thimble with petroleum ether (B.P. below 40° C.). The extraction was carried out in a Soxhlet Extraction Apparatus with petroleum ether and was continued for some 20 to 24 hours. The ethereal extract was then filtered into a weighed flask and the ether distilled off on a water bath and the oil freed from any remaining ether and dried in a steam oven at 98–99° C. Every half hour the flask was cooled in a desiccator and weighed. The series of weighings thus obtained showed first a decrease as ether and moisture were expelled, and then an increase as the oil slowly oxidised. The smallest weight was taken as the true weight of the dry oil. Duplicates were carried out in all cases and invariably were in close agreement; the greater number being well within 0·25 %.

The figures given (Table I) indicate that linseed grown in England is by no means inferior in oil to any of the imported samples. Indeed, the results as a whole compare by no means unfavourably with the figures given by Leather[1] for 52 samples of Indian linseed grown in all parts of India, in spite of the fact that the climate of India is generally considered to be especially favourable to the growth of oil-bearing crops.

Summary of Leather's results for Indian Linseed.

District	Aver. % of oil	Varying between
Punjab	38·27 (aver. of 10)	35·6 % and 41·91 %
Central Provinces	41·36 („ 21)	36·47 „ 44·20
Bombay Presidency	40·71 („ 7)	41·23 „ 44·45
Madras Presidency	40·12 („ 5)	40·46 „ 41·71
United Provinces	42·58 („ 9)	41·44 „ 44·55

The crops grown at Wye gave consistently higher figures than those at the other centres, from which it would appear that linseed is more suitable as a south country crop, doing best under the warmer more equable weather conditions of our southern shores. If this is so, one would expect similar high figures for the linseed grown at the Seale-Hayne Agricultural College (the Devonshire Centre). Here, however, the season was a very wet one, the yield was low (in one case less than a hundred-weight to the acre), and great difficulty was experienced in

[1] Leather, *loc. cit.*

harvesting and drying the crop. The seed was black and of poor quality and had a distinct "musty" odour; and it was a matter of some surprise that the oil content was as high as it was actually found to be.

Variety to Sow.

Many so-called varieties of linseed are cultivated at the present day and they exhibit differences sufficiently well marked for them to be classified by some authorities as varieties of different species. It is probable that in some cases they are not real botanical varieties at all but rather "economic" ones brought about by long continued cultivation in different climates and different methods of treatment. These differences, however, persist for a reasonable period when the crop is grown away from its natural environment, and this being so, linseed grown in or exported from any particular region is generally called a variety if it shows any well marked and fairly persistent characteristic.

In deciding which are the most profitable varieties to grow, not only the percentage of oil, but also the yield per acre must be taken into account. In other words we should be able to express the return of oil per acre before we can effect a strict comparison between the different varieties. And this comparison should be made between the more commonly grown varieties in as many different localities as possible and during several seasons before any certain conclusions can be drawn. In the present communication we have endeavoured to obtain some information on the point in the case of those varieties which were grown in 1913 at four of the Centres already mentioned (*vide* Tables II A and II B). By multiplying the percentages of oil in the samples by the yields in cwts. per acre and dividing the products by 100 we obtain the yields of oil per acre, and the numbers so obtained afford some indication of the relative merits of the different varieties dealt with. Considering the wide variations in yield and the great differences in the quality of the seed grown at the different centres, the results as set forth in the accompanying tables are remarkably consistent and bring out the relative merits of the different varieties in a very striking manner. Plate seed comes an easy first, Steppe seed a moderate second, Moroccan third, while Dutch is the poorest of the four; except in the case of the Harper-Adams crops of which the Plate and Moroccan varieties do not appear to have done as well as was the case at Wye and Camblesforth. It is noticeable that even the very poor crops from the Seale-Hayne centre still indicated the marked superiority of the Plate seed over the other varieties as an oil-producing crop.

TABLE II A.

Plot No.	Type of sowing	Yield, cwts per acre	Average yield thickly sown plots	Average yield thinly sown plots	Total average yield	Percentage oil	Yield of oil per acre, percentage oil × yield ÷ 100	Oil per acre when Plate = 100	Relative order
Moroccan 10*	thick†	12 cwt. 20 lbs.	14 cwt. 44 lbs.	.. .. ..	12 cwt. 25 lbs.	.. ..	6·1775 cwt.	62·5	3
15‡	,,	16 ,, 64 ,,				42·9	5·2435 ,,	83·2	3
11*	thin§	8 ,, 16 ,,	.. .. ..	10 cwt. 8 lbs.		.. ..	4·3200 ,,	98·5	2
13‡	,,	12 ,, 0 ,,	.. .. ..						
Dutch 1*	thick	11 ,, 2 ,,	12 cwt. 46 lbs.	.. .. ..	11 cwt. 86 lbs.	.. ..	4·6785 ,,	62·3	4
7‡	,,	13 ,, 68 ,,				37·7	4·0515 ,,	74·6	4
3‡	thin	13 ,, 12 ,,	.. .. ..	11 cwt. 14 lbs.		.. ..	4·1920 ,,	66·9	4
4*	,,	9 ,, 16 ,,	.. .. ..						
Plate 9*	thick	14 ,, 64 ,,	14 cwt. 72 lbs.	.. .. ..	14 cwt. 70 lbs.	.. ..	6·2670 ,,	100	1
16‡	,,	14 ,, 80 ,,				42·8	6·2595 ,,	100	1
12*	thin	10 ,, 0 ,,	.. .. ..	14 cwt. 70 lbs.		.. ..	6·2575 ,,	100	1
14‡	,,	19 ,, 24 ,,	.. .. ..						
Steppe 6*	thick	11 ,, 40 ,,	13 cwt. 4 lbs.	.. .. ..	13 cwt. 53 lbs.	.. ..	5·4095 ,,	74·2	3
8‡	,,	14 ,, 80 ,,				41·5	5·6150 ,,	89·7	2
2*	thin	13 ,, 4 ,,	.. .. ..	13 cwt. 98 lbs.		.. ..	5·7685 ,,	92·2	2
5‡	,,	14 ,, 88 ,,	.. .. ..						

* These plots were very poor chalk soil; plots 6 and 12 being especially poor. On these plots the crop was noticeably poor and thin.

† *i.e.* 112 lbs. per acre.

‡ These plots were alluvium and had been prepared for potatoes and were in a better and more sheltered situation than the chalk plots. The differences in soil and situation may possibly account for the anomalies which are noticeable in the yields from the different plots. This will be dealt with at length in a "Report on Linseed Experiments for 1913."

§ *i.e.* 70 lbs. per acre.

TABLE II B.

Variety	Yield	Per cent. oil	Oil per acre	Oil per acre (Plate = 100)	Relative order
	cwt. lbs.				
Camblesforth					
Plate	6 12	39·69	2·423 cwt.	100·00	1
Moroccan	5 67	40·13	2·247 „	92·45	2
Dutch	4 74	35·08	1·635 „	84·93	3
Scale-Hayne					
Plate	3 63	37·72	1·345 „	100·00	1
Moroccan	1 21	39·06	3·977 „	29·55	2
Dutch	0 106	34·60	3·274 „	24·33	3
Harper-Adams					
Plate	8 97	41·35	3·667 „	100·00	2
Moroccan	8 67	40·86	3·513 „	95·80	3
Dutch	9 74	36·71	4·465 „	121·76	1

Relation between oil content and size of seed.

Leather[1] when examining Indian linseeds, states that he could get little evidence that any connection exists between the size of the seed and the percentage of oil they contain. Some of the large kinds from the Central Provinces weighing 8·0 grm. per 1000 seeds were found to contain only as much oil as one grown at Bilaspur (in the Central Provinces) which weighed only 5·5 grm. per 1000 seeds. His comparisons, however, are not strict ones, for the Central Provinces and the other regions of India from which he obtained his seeds are vast areas, over which very varied climatic conditions obtain, and in many cases they produce types of linseed exhibiting well marked characteristics. It is highly probable that in these instances any correlation of size of seed with oil content would be hidden by the far greater variations due to variety and varying climatic conditions under which they were grown. That this may be so is evident from a comparison of the oil content and size of seed grown in different parts of Bombay Presidency, a region throughout which the climate is more uniform than is the case with the other regions of India.

Weight of 1000 seeds	Oil content
6 to 7 grm.	41·23 % (single sample)
7 to 8 grm.	42·22 % (average of 3 samples)
8 to 9 grm.	43·83 „ „ „ „

These show a distinct, though slight, increase of oil content with increase in size of seed. Our own experience with both English grown

[1] *Loc. cit.*

and imported seed goes to support such a relationship. From Table III it will be seen that by comparing either the imported or the home grown varieties with one another, very little regularity can be observed. It is evident that in this case the variations in oil content due to difference of variety mask any difference due to varying size of seed. Very different, however, is the case when we compare each imported variety with the seed produced *from the same sample* grown in England. Here a regularity is noticeable: in practically all cases an increase in oil content is accompanied by an increase in size of seed. Strict proportionality between the two could not of course be expected from the very nature of the case, but that there is a parallelism between them, other things being equal, seems to be sufficiently brought out by the figures given in the table below.

TABLE III. *Showing Relation between Oil Content and size of Seed of Different Varieties of Linseed Grown under Different Conditions.*

Variety of seed	Imported		English grown	
	Oil content	Wt. of 1000 seeds	Oil content	Wt. of 1000 seeds
Pskoff	37·45 %	4·198 grms.	40·55 %	4·484 grms.
Moroccan	40·60	10·166 „	42·90	13·098 „
			40·13	13·538 „
			39·06	11·132 „
			40·86	13·392 „
Plate	38·45	6·108 „	42·80	8·840 „
			39·69	9·204 „
			37·72	7·712 „
			41·35	8·744 „
Dutch	35·49	4·817 „	37·69	5·410 „
			35·08	4·810 „
			36·71	5·164 „
			34·60	4·066 „
			34·08	3·904 „
Dutch	38·65	4·754 „	38·30	4·861 „
Dutch	36·13	4·599 „	37·11	5·252 „
Steppe	38·90	5·076 „	41·50	7·198 „

Frequent Change of Seed.

In Russia, the country from which the best flax seed is obtainable, change of seed is not an agricultural consideration. The crops are almost invariably grown from seed of the previous harvest and in many cases the farmers have had their seed in the family for more than 20 years[1].

In all other European countries, however, emphasis is laid upon the necessity of frequently changing *flax seed*, and the same practice has been recommended in the case of linseed. It is not known definitely, however, whether continuous growing from seed of the previous year's crop has any effect on the oil content of the seed and in this connection very few data are available. In one case[2] Riga seed was sown in Essex in 1911 and the oil content of the resulting seed was 35·66 %. The seed from this crop was sown in 1912 when it produced seed containing only 26·73 % of oil. Such a decrease (nearly 9 per cent.) is, however, very unusual whatever the cultural conditions may be and it is probably due to some other cause than the one in question. Personally, we have never come across any sample of seed with such a low oil content as this; the lowest we have met with was in a sample discovered by one of us growing wild in the south of Ireland and consisting of very small seed with a particularly hard and thick seed coat. This had an oil content of 29·07 %.

The only other data available, as far as we have been able to discover, are some given by Leather. He grew specimens of linseed rich in oil, obtained from various parts of India, at Lyallpur in the Punjab at farms where seed of poor quality was generally produced and, as the subjoined table shows, found a small but continuous decrease in oil throughout two years.

	Orig. seed (1904)	Produce of 1905	Produce of 1906
White Linseed from Cawnpore	44·62 %	41·28 %	39·90 %
" " " Khandwa	44·96	44·18	42·93
Brown " " " Damoh	45·34	43·07	43·57
" " " Partabgarh	43·17	40·98	38·31
" " " Cawnpore	42·05	40·97	39·43
" " " Sholapun	41·13	40·42	38·82

[1] J. V. Eyre, Sup. to *Journal Bd. of Agric.* No. 12, Jan. 1914, p. 17.

[2] *Journal Bd. of Agric.* Vol. XX. No. 5 (Aug. 1913), p. 381.

In this case, however, the crops were grown on farms which produced poor quality linseed only and hence the conditions were all in favour of a decrease in oil content.

Up to the present we have not had the opportunity of testing this in many cases, but a few data have been obtained with Pskoff and Plate seed grown for several successive generations in different parts of England.

The results are given in Table IV.

TABLE IV.

Pskoff seed.	Imported 1911	37·45 % oil
,,	Grown Wimbledon (1912) from 1911 imported seed ..	35·65 ,,
,,	Grown Wye (1913) from Wimbledon 1912 seed	33·21 ,,
,,	Grown Wye (1914) from Wye 1913 Wimbledon 1912 seed	33·35 ,,
,,	Grown Wye (1913) from imported (1911) seed	34·05 ,,
,,	Grown Wye (1914) from Wye 1913 seed	34·96 ,,
,,	Grown Wye (1914) from imported (1911) seed	33·78 ,,
,,	Grown Selby (1913) from imported (1912) seed	36·68 ,,
,,	Grown Selby (1914) from Selby (1913) seed	35·05 ,,
Plate seed.	Imported (1912)	38·45 ,,
,,	Grown Wye (1913) from imported (1912) seed	42·82 ,,
,,	Grown Wye (1914) from Wye (1913) seed	41·64 ,,
,,	Grown Wye (1914) from imported (1913) seed	39·10 ,,

It will be seen that in some instances a diminution in oil content does occur from generation to generation and there are indications that the percentage of oil produced eventually becomes more or less constant. On the other hand such diminution might conceivably be due to seasonal and cultural variations or to variations in soil factors from year to year.

Such effects could be eliminated by growing parent, child, grandchild, and great-grandchild etc. seed during the same season and on the same plots. This has been done in one or two instances and as shown in the table the variations then disappear; practically no difference in oil content being exhibited by the various generations when grown side by side in the same season and under identical conditions of soil and cultivation.

At any rate our results give no support to the view that repeated growth of linseed from the same stock gives rise to a seed of diminished oil content.

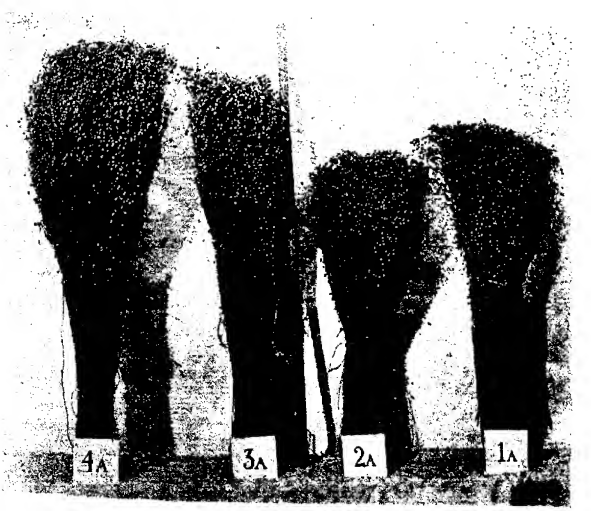
The effect of artificial manures on the oil content.

The use of artificial manures has been found to occasion only a very slight variation in the oil content of linseed. This conclusion is based upon the results of trials carried out with Dutch seeds at Rothamsted during the hot season of 1911 and at Holmes Chapel Agricultural

College during 1913 (cf. Table V). As will be seen from the table in no case was there a difference in the total oil content of more than 1·6 %: a difference which is of but slight economic importance. The main effect produced by the artificial manures has been found to be in the direction of influencing the yields both of seed and straw as may be seen from the following illustration.

TABLE V. *Showing effect of Manuring on the Oil Content of Linseed.* (Dutch white flowering.)

Holmes Chapel				Rothamsted—Hoos barley field		
Plot No.	Type of sowing	Manuring	Oil content	Plot No.	Manuring	Oil content
1	Thin	none	33·22 %	1 A	N	34·45 %
2	,,	N	33·57	2 A	N+P	34·11
3	,,	N+P	33·45	3 A	N+K	34·25
4	,,	N+P+K	34·08	4 A	N+P+K	34·67
5	Thick	none	34·47			
6	,,	N	34·79			
7	,,	N+P	34·35			
8	,,	N+P+K	34·53			



Dutch white flowering linseed, grown at Rothamsted.

It has been found at some centres that it is the combination of superphosphate and potash that brings about the most striking differences of yields; and it is interesting in this connection that the only significant increase in oil content due to manurial treatment is brought about by the same combination. At other centres owing, presumably, to unfavourable condition of experiment the application of artificial manures seems to have had little or no effect in increasing the yield of the crop. This point, however, cannot be dealt with here but, together with other considerations of a purely cultural nature, is reserved for a separate report.

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